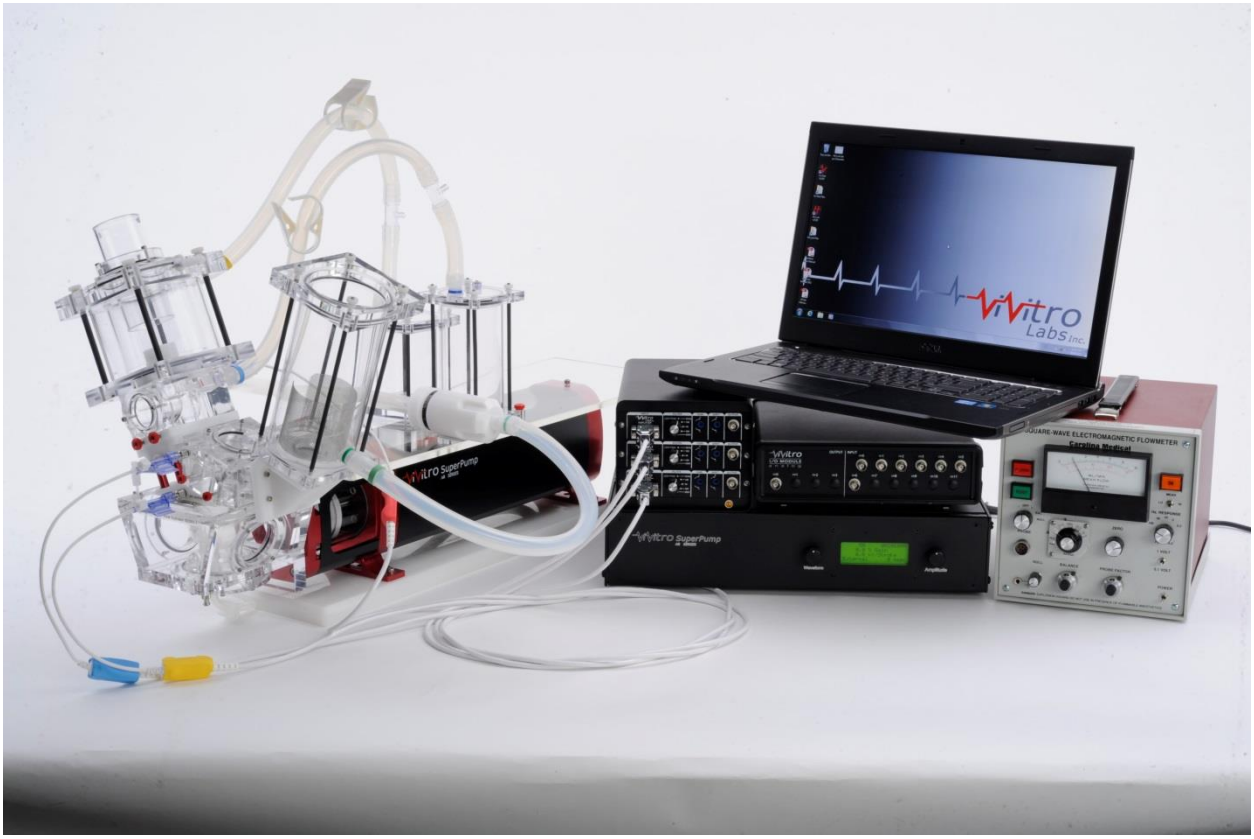
Pulse Duplicator System
User Manual



Pulse Duplicator System Including Data Acquisition System

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ViVitro Labs Pulse Duplicator System
Designed and Distributed by ViVitro Labs Inc., BC, Canada

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1. About ViVitro Labs

ViVitro Labs Inc. offers industry leading cardiovascular test equipment and related laboratory testing services. Hundreds of organizations in over 39 countries for 30+ years have trusted ViVitro's expertise, accuracy, and quality for their cardiovascular device testing.

As the developer of the world's first Pulse Duplicator, the ViVitro name has been synonymous with cardiovascular device testing equipment. ViVitro hardware and software products have been used by leading R&D facilities and academic labs worldwide, and its equipment and testing methods being cited in hundreds of peer reviewed publications http://ViVitrolabs.com/company/cited-publications/. The ViVitro Labs' Pulse Duplicator System (in particular the ViVitro Labs SuperPump) has become a worldwide standard to simulate physiological heart conditions in academic research applications.

ViVitro products are manufactured by StarFish Medical in an ISO 13485:2003 certified manufacturing facility. ViVitro Labs Inc., also holds ISO/IEC 17025 accreditation for laboratory testing services endorsed by A2LA and based on ISO 5840. This scope of accreditation includes the physical and mechanical testing of heart valve substitutes including durability, hydrodynamic, and flow visualization testing.

ViVitro Labs is widely recognized as the authority on cardiovascular device testing. ViVitro Laboratory Services are engaged worldwide for hydrodynamic, durability, and flow visualization testing of heart valves and other cardiac devices. ViVitro has been the trusted name in regulatory approvals for over 30 years and is renowned for its proven success from product development testing through to full regulatory submission.

ViVitro Labs is a member of the ISO 5840 standards committee and is actively engaged in developing regulatory requirements. Leveraging this intimate knowledge of the standard, ViVitro Labs ensures that test protocols will meet ever changing regulatory requirements. ViVitro's Laboratory Testing Services offers an ISO/IEC 17025 certified lab using ViVitro equipment, to conduct 3rd party independent testing. ViVitro's accredited testing lab is governed by a mature Quality Management System (QMS) certified to meet the ISO 5840. This gives assurance to stakeholders and regulatory bodies that results are obtained by qualified personnel using traceable calibrated equipment and up to-date test methods, all supervised by a quality assurance department.

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1.1 Cautions and Warnings

- **After you receive your Pulse Duplicator, ensure that you let the electrical enclosures sit indoors at room temperature for at least one day before plugging them in.**
- The systems must not be used in environments at higher than 75% humidity.
- Read all of the instructions before operating the SuperPump
- This instrument is intended for research use only and must be operated by trained lab personnel.
- To ensure adequate cooling of the SuperPump controller and pump, place on a clean, flat surface, and be sure that there is at least 10 cm (4 in) of clearance around each side of the controller and motor end of the pump. Make sure that the fan vent is not blocked.
- Always connect the power supply to a 3-prong, grounded AC outlet rated 4A, 110–240 V using the AC power cord provided with the SuperPump. Do not use an adapter to an ungrounded outlet. Before plugging the instrument in, be sure that the correct fuses are installed. See section 3.9.
- Electrical devices pose the risk of electric shock. To reduce the risk of shock, do not open any covers that are fastened with screws.
- While SuperPump is designed to withstand spills on its exterior surface, do not allow fluids to enter the interior of the instrument. In the event of such a spill, disconnect the power cable before cleaning up.
- Do not operate SuperPump in extreme humidity or in conditions that can create condensation. Protect the instrument against dust and moisture, and avoid physical shock and strong forces.
- Clean the controller and pump exterior with a dry cloth. Disconnect power before cleaning
- Use caution when moving the controller and/or pump. Disconnect power and pump cable before moving the system to a new location.
- Do not use the SuperPump system if there is evidence of physical damage or if the system has been dropped or subject to impact.
- Do not attempt to service the product beyond what has been described in this manual. All other servicing should be referred to qualified service personnel.

1.2 About this Manual

This document describes the Pulse Duplicator System's procedures for:

1. Assembly of the system's hardware.
2. Connection of the system's electrical components.
3. Calibration and system setup.
4. Operator procedures for the system's daily use.
5. Operator procedures for ViViTest Software.
6. Operator procedures for ViViGen Software.
7. Optional valve and other cardiac structure mounting procedures for use within the Model Left Heart.

NOTE: The user may opt to manipulate the system to achieve alternate states/results/configurations. Should the user carry this out it should be noted that the pressures and flows are kept within the limitations of the system to ensure the system is not overloaded.

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2. About the Pulse Duplicator System

2.1 System Hardware

The ViVitro Labs Inc. Pulse Duplicator System assesses heart valve function under simulated cardiac conditions. The system simulates the function of the heart's ventricle which generates pulsatile flow through a prosthetic heart valve in a ViVitro Labs Model Left Heart. A Pulse Duplicator simulates physiological or other complex pressure variations which (when connected to the ViViTest software via sensors) provides detailed data return on prosthetic heart valve(s) and cardiac system performance.

The ViVitro Labs Pulse Duplicator System is ideal for testing a variety of prosthetic heart valves. Standard and optional hardware components facilitate the mounting of mechanical, stented and stentless bioprostheses, percutaneous devices. The system is also suitable for other related cardiovascular purposes such as measurements of root compliance (in conjunction with video recording) or average coronary flow.

2.2 Hardware Features

The Pulse Duplicator System from ViVitro Labs Inc. features include:

- Assessment of heart valve performance and function under simulated cardiac conditions.
- View and monitor heart valves under simulated conditions via:
    - transparent parts and view ports to observe and video record valve function and adjacent flow fields;
    - aids in flow visualization studies;
    - thin acrylic windows permit ultrasound transmission for interrogation of flow fields in aortic and mitral regions;
    - transducer sites to measure wall pressures in the atrium, ventricle, aortic outflow tract, downstream of the aortic valve and upstream of the aortic and mitral valves.
- Control pulsatile fluid flows to simulate various cardiac flow conditions to include arrhythmia, normal, hypo and hypertensive states at various cardiac outputs and beat rates.
- Peripheral resistance and supplementary characteristic compliances to simulate cardiovascular character and resistance.
- Control, create, download and playback cardiac waveforms at various physiological states and lower frequencies.
- Quality data acquisition which meets verification and testing in accordance with ISO 13485 requirements and aids in the collection of data to meet ISO 5840 to FDA requirements through the use of ViViTest software.
- Optional components enable:
    - Testing of various cardiac valve types and cardiac structures.
    - Viscoelastic simulated behavior to produce realistic ventricle pressure waveforms.
    - Operate test fluid at 37ºC to simulate physiological conditions.

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2.3 ViViTest Software

This document describes the ViViTest software program designed to acquire data from the ViVitro Labs Pulse Duplicator System in characterizing heart valves. This document along with hardware operations manuals for the various elements of the Pulse Duplicator System should be considered as the primary reference document for operations of the ViViTest software package.

ViViTest is designed for monitoring, acquisition and analysis of data generated by laboratory systems for assessment of heart valves. This software is primarily developed for the Pulse Duplicator System from ViVitro Labs. For use with other systems please contact ViVitro Labs for technical assistance.

2.4 Software Features

The software consists of four primary processes:

- Waveform Acquisition
- Waveform Analysis
- Waveform Comparison
- Waveform Review

and one secondary utility:

- Sensor Calibration

The ViViTest software is shipped pre-installed on a PC. Components:

- Laptop or Desktop PC (with monitor, keyboard and mouse)
- I/O Module
- .NET 3.5 framework

2.5 Hardware System

The ViViTest software is shipped pre-installed on a PC. Components:

- Laptop or Desktop PC (with monitor, keyboard and mouse)
- I/O Module
- .NET 3.5 framework

The ViViTest software application is designed to run on the following minimum hardware specifications:

- LCD monitor capable of 1600 x 900 resolution
- High throughput video card
- I/O Module
- 2GB RAM
- 38GB Hard drive
- Pentium 4 3.6 GHz Processor (single core) or better

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2.6 Software Requirements

For the software to run smoothly the following software is required to be installed on the computer:

- Microsoft Windows XP Professional SP3, Windows 7, Windows 8
- .NET 3.5 framework.

2.7 Upgrade Installation Instructions

As the software upgrades are released you will be notified via email.
Instructions:

1. Download updater installer file.
2. Select the single comprehensive installer executable.
3. An installation Wizard will guide you through the installation steps.
4. Unless provided the installation key should be left blank.

NOTE: With some upgrade releases there may be the necessity to upgrade the I/O Module files and/or the .NET files. The Wizard will guide you through this. Should you incur problems, contact ViVitro Labs directly.

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3. About the AR Series SuperPump

3.1 System Hardware

The Advanced Research series SuperPump System made by ViVitro Labs Inc. consists of a digital amplifier with stroke volume display and preprogrammed waveforms driving a piston-in-cylinder pump. The pump piston is located by a ball screw/servo motor/encoder unit driven by a motor controller which compares actual position against the desired position defined by the input waveform. The precision of the ball screw and the resolution of the encoder ensure accurate positioning of the piston resulting in the desired pulsatile flow.

The controller can be used stand alone by selecting one of the built in waveforms or an external signal can be input. ViVigen software is included with the pump to allow the generation of custom waveforms which can then be uploaded to the controller via the built in USB port. The SuperPump is designed for use as part of the ViVitro Pulse Duplicator and includes the required inputs and outputs for this. These same I/O including analog outputs for position, dL/dt (flow) and the sync pulse make the SuperPump adaptable to any custom test apparatus. The ViVitro Labs AR series SuperPump is ideal for testing a variety of cardiovascular devices.

3.2 Features

The SuperPump-AR series from ViVitro Labs Inc. features include:

- Digital precision control of a pump-in-cylinder designed linear actuator used to generate physiological flows;
- Stand-alone capability with five (5) preprogrammed cardiac waveforms created by user;
- Range of capable rates are 4 to 200 beats per minute;
- Range of stroke volume 0 to 150 ml;
- Digital controlled and created waveforms by ViViGen or ViViTest software;
- Control, create, input or store cardiac waveforms at various physiological states and frequencies through ViVitro software or other std waveform generating equipment;
- Manufactured by an ISO 13485 accredited facility designed to meet regulatory requirements (ISO 5840 and FDA) or research needs.

NOTE: The user may opt to manipulate the system to achieve alternate states/results/configurations. Should the user carry this out it should be noted that the pressures and flows are kept within the limitations of the system to ensure the system is not overloaded.

3.3 SuperPump Initial Set-up Procedures

The SuperPump System has several steps to the main mechanical sub-assemblies, and a few steps for the electrical components installation/configuration.

NOTE: It is critical for the assembly steps to be followed in order to assure successful operation.

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3.4 Unpacking – List of Components

The SuperPump AR arrives in 2 containers; a wooden shipping crate for the pump and a cardboard container for the controller. Inspect the shipping containers for external damage. Retain the shipping carton and packing materials for possible re-shipment. Inspect the system components for damage. If there is evidence of damage contact the carrier, initiate a damage claim, and inform ViVitro Labs.

The SuperPump arrives pre-assembled with the following parts:

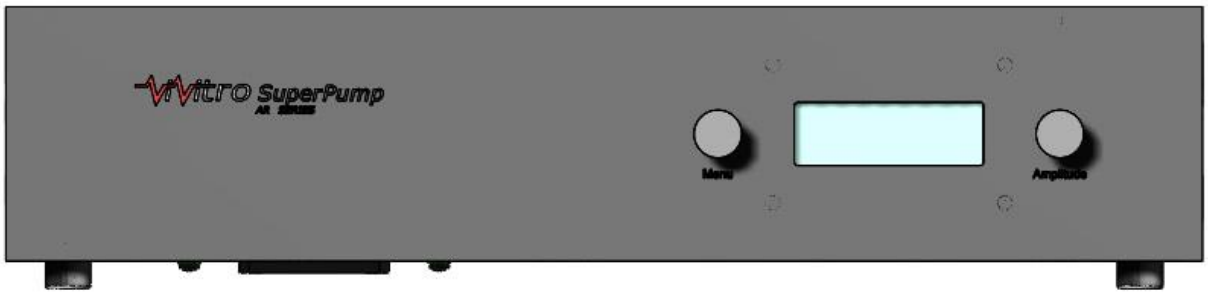
- Mounting base
- Linear Actuator with Cable
- Standard Pump Head
- Actuator Extension Cable
- SuperPump Controller
- USB flash drive with ViVigen software

3.5 Installing the SuperPump

The SuperPump is supplied mounted to a rigid plastic base for use on a bench top. Optionally the pump may be mounted directly to a structure or test apparatus using #10-32 fasteners through the mounting feet of the pump. See bolt pattern layout in Section 4.9. It is recommended that the SuperPump be mounted horizontally. The back side of the pump cylinder is provided with a drain, however vertical mounting of the pump can result in water entering the ball screw/motor causing an electrical hazard.

The controller should be located away from the pump to avoid any water or test fluid. An extension cable is provided to allow extra separation if required. It is recommended that the pump act on the test fluid through a membrane to maintain isolation between working and test fluid. The piston should not be directly exposed to blood analog solution or saline. Distilled water is recommended as the working fluid.

3.6 Front Panel



The front panel consists of 2 controls and the display. The controls are rotary encoders with push switches. To the left of the display is the menu knob which allows scrolling by turning the knob and selection by pressing the knob. To the right of the display is the amplitude knob which adjusts the amplitude of the output waveform and starts and stops the pump by pressing the knob

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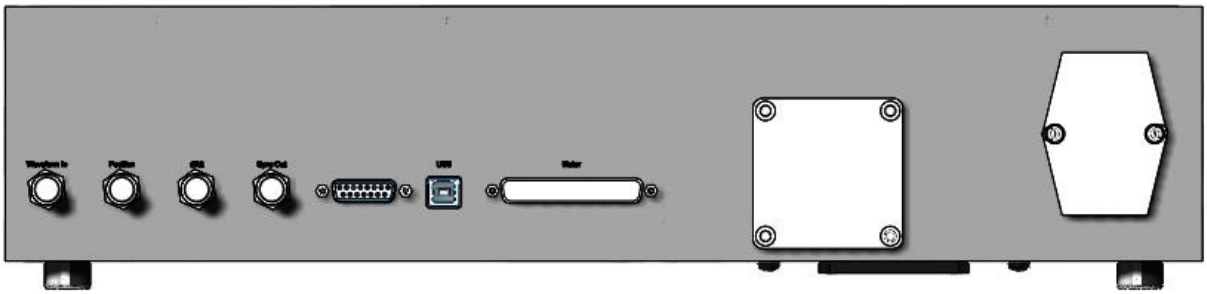
3.7 Rear Panel



The rear panel contains all of the connectors. From left to right:

- **Waveform In**: BNC connector used when an external signal is used as the waveform
- **Position**: BNC connector analog output signal for the piston position
- **dL/dt**: BNC connector analog output signal proportional to instantaneous flow rate
- **Sync Out**: BNC connector 5V TTL signal triggered at the rising zero crossing of the waveform. The timing of the signal can be adjusted to any point in the waveform
- Troubleshooting port : no user accessible functions
- **USB**: For uploading custom waveforms created using ViVigen software or for use with ViVitest when the SuperPump is used as part of the Pulse Duplicator
- **Motor**: Cable connection to the pump
- Cooling fan
- Power input: Power cord, fuse holder and main power on/off

3.8 Electrical Requirements

ViVitro Labs Inc. will endeavor to have your system's components adapted to meet your country's power supply, however, as an extra precaution, it is advised that all equipment designated to be connected to a power supply be checked prior to use. Use only the power cord supplied with your SuperPump. The controller must be plugged into a grounded outlet rated for 300W.

3.9 Fuses

Two fuses are supplied and required for the SuperPump Controller. They are located in the fuse drawer on the power input module on the back of the controller. At 230VAC use two 2A, 5mm x 20mm 230V fuses. At 120VAC use two 4A, 5mm x 20mm 230V fuses. Contact ViVitro Labs if you require replacement fuses.

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4. Running the SuperPump System

4.1 Waveform Source

The SuperPump may be operated using either:

a) USB connection to a computer running ViViTest Software – see ViViTest user manual
b) Internally stored waveform – see section 8 ViVigen
c) Externally generated waveform

If an external waveform is to be used, plug an appropriate BNC cable from the waveform source to the Waveform In port on the back of the SuperPump Controller (sec 2.4).

The external waveform should conform to the following specifications:

- Amplitude: 10.0 Vpp (±5.0 Vpp) maximum. Any waveform that exceeds this amplitude will not be accurately represented in the pump movement.
- Rate [Frequency]: 4 bpm (beats per minute) minimum to 200 bpm maximum. [0.067 Hz to 3.33 Hz]

NOTE: Do not input waveforms with frequency greater than the listed maximum.

- Form: Bi-polar. The waveform should be centered about 0 V (ground), without D.C. offset, unless offset is intended for recreating a particular wave shape. The peak-to-peak limits with offset must still remain within ±5.0 Vpp relative to ground.
- Noise and Distortion: both noise and distortion will affect the accuracy of the pump movement; therefore the signal source should be clean, with THD+N (total harmonic distortion and noise) below 5%.

Note: the maximum velocity of the piston is limited to approximately 60mm/sec. As amplitude and frequency are increased the velocity will also increase. If the pump cannot match the desired waveform a pump error will result. In this case either the maximum slope of the waveform must be reduced or the frequency or amplitude must be reduced.

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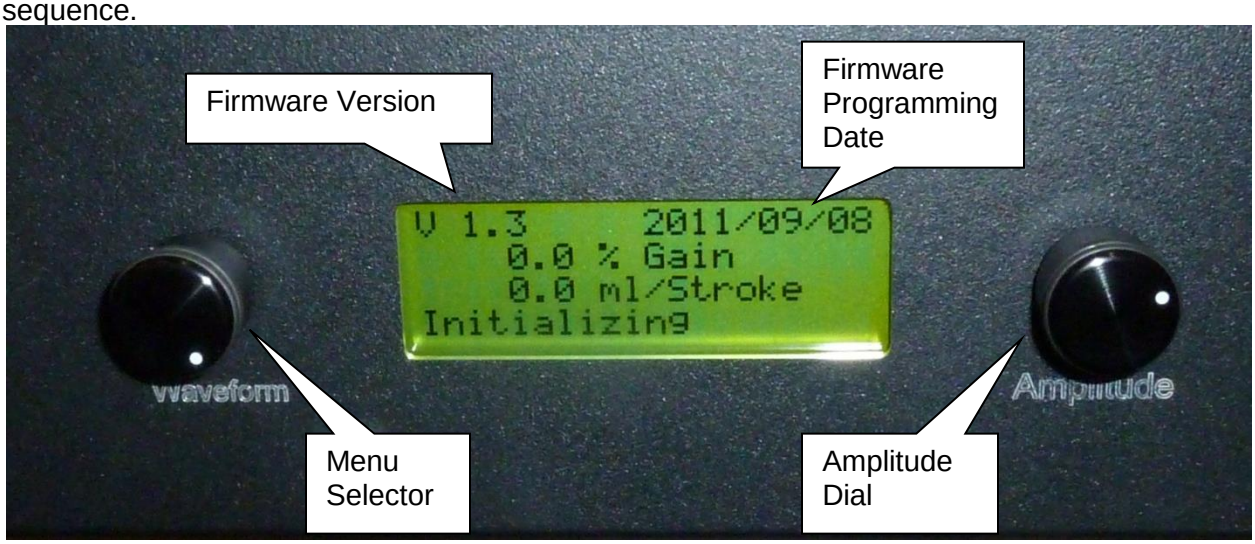
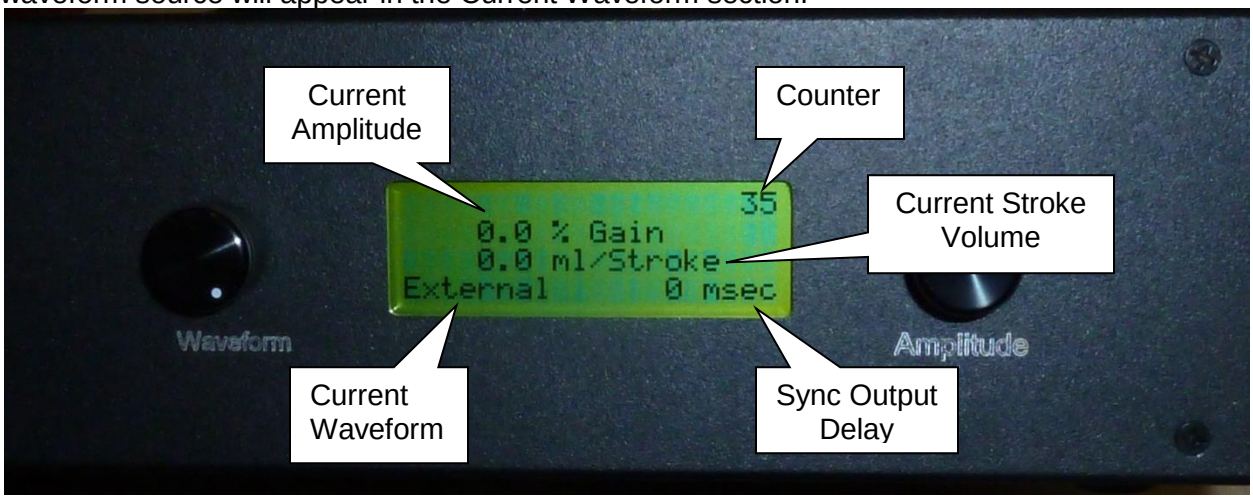
4.2 Initialize System

Turn on the power switch on the back of the Controller. The screen on the front of the Controller will light up and indicate that it is Initializing. It will then automatically begin a Homing Pump sequence.



After automatically Homing Pump, the screen should appear as below. The most recently used waveform source will appear in the Current Waveform section.



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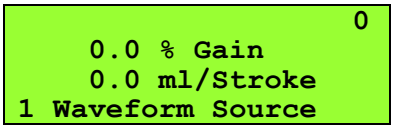
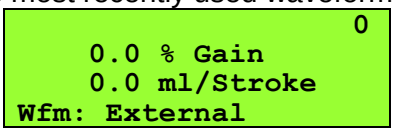
4.3 Select Waveform

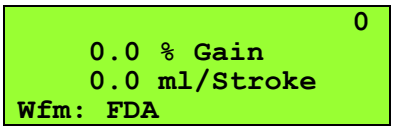
Press the Menu Selector Dial. Turn the Menu dial until “1 Waveform Source” appears as below.



While the message Waveform Source is on the screen, press the dial again. The screen will now indicate the most recently used waveform source.

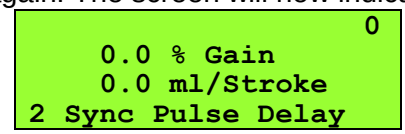


If the current waveform source is desired press the dial again to escape the waveform selector. If a stored waveform is desired turn the dial clockwise and press the dial when you have chosen the correct waveform.
Refer to Section 9 to load standard waveforms, edit, or create waveforms and load them onto the SuperPump Controller.



4.4 Set Sync Delay

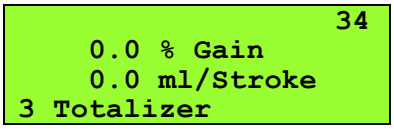
Press the Menu Selector Dial. Turn the menu knob until “2 Sync Pulse Delay” is on the screen, press the dial again. The screen will now indicate the current sync delay in msec.



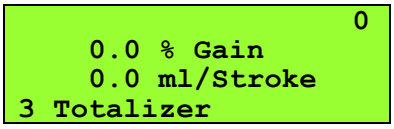
To adjust the delay turn the dial until the correct delay is displayed and then press the dial.

4.5 Change the Cycle Count

Press the Menu Selector Dial. Turn the menu knob until “3 Totalizer” is on the screen, press the dial again.



To adjust the counter turn the dial until the correct value is displayed and then press the dial.



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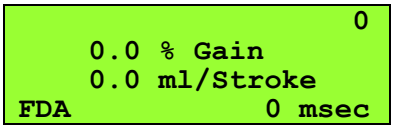
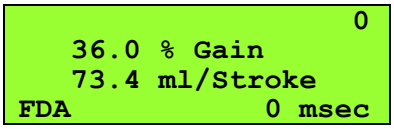
4.6 Pump Operation



Turn the Amplitude Dial until the desired stroke volume is reached. For example:

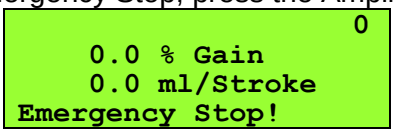


To stop the pump either turn the Amplitude Dial to 0 or press the Amplitude Dial. In this case an Emergency Stop alarm will appear. To clear this alarm, press the Amplitude Dial. The pump will automatically perform a Homing Sequence.

4.7 SuperPump Faults and Alarms

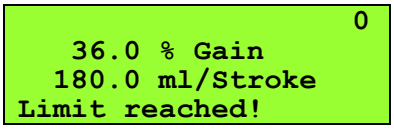
4.7.1 Emergency Stop

If case of an Emergency Stop, press the Amplitude Dial to clear the alarm and Home the pump.



4.7.2 Limit Switch

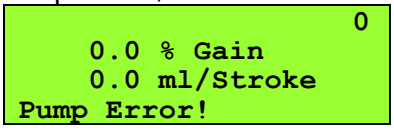
The SuperPump actuator contains a factory-set limit sensor for the maximum push and pull stroke. In case of a Limit reached alarm, press the Amplitude Dial to clear the alarm and Home the pump.



4.7.3 Pump Error

If the Controller loses communication with the Pump or if the Pump is unable to move, a Pump error message will appear. If this occurs,

1. turn off the system
2. check that the cables are properly installed
3. ensure that the pump is free of mechanical interference and damage
4. turn the controller on and allow it to initialize normally
5. If the alarm persists, contact the manufacturer



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4.8 Specifications

Pump	
Pump bore diameter	Nominal 69.85mm (2.750”)
Pump stroke	0-40mm (±20mm)
Displacement	0-150mL
Rate	4 to 200 beats per minute
Inputs	
External Waveform	0-10 Vpp 0.067 Hz to 3.33 Hz (4 to 200 beats per minute)
Outputs	
Position	-5 to 5 V (200mV/mm)
DL/dt	-5 to 5 V (539 ml/s/V)
Sync	+5V TTL LE at rising zero crossing
Power Requirements	
Voltage	90-240 VAC / 50-60Hz
Power	Max 300W
Dimensions	
Controller	11 x 48 x 33 cm
Pump	15 x 55 x 17 cm

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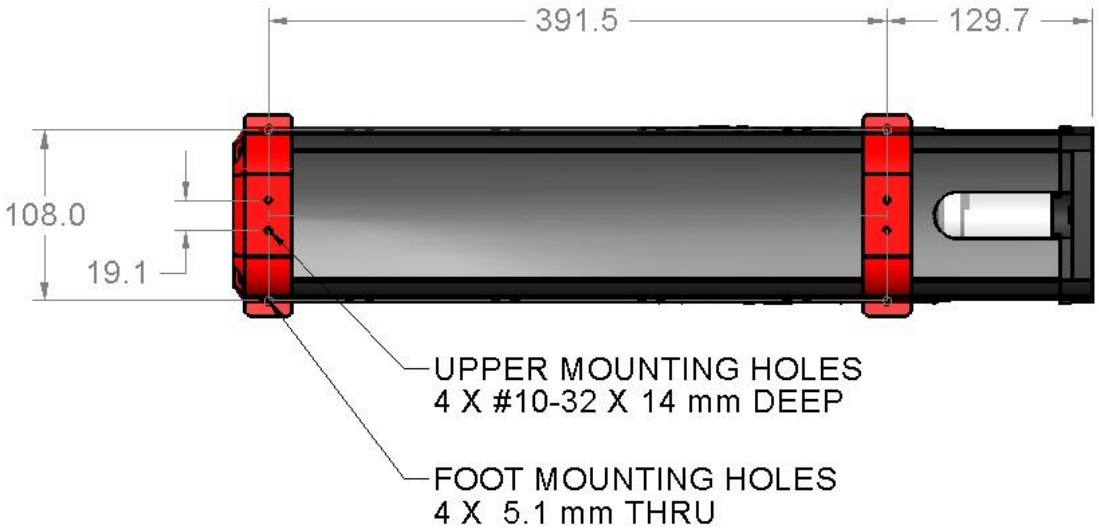
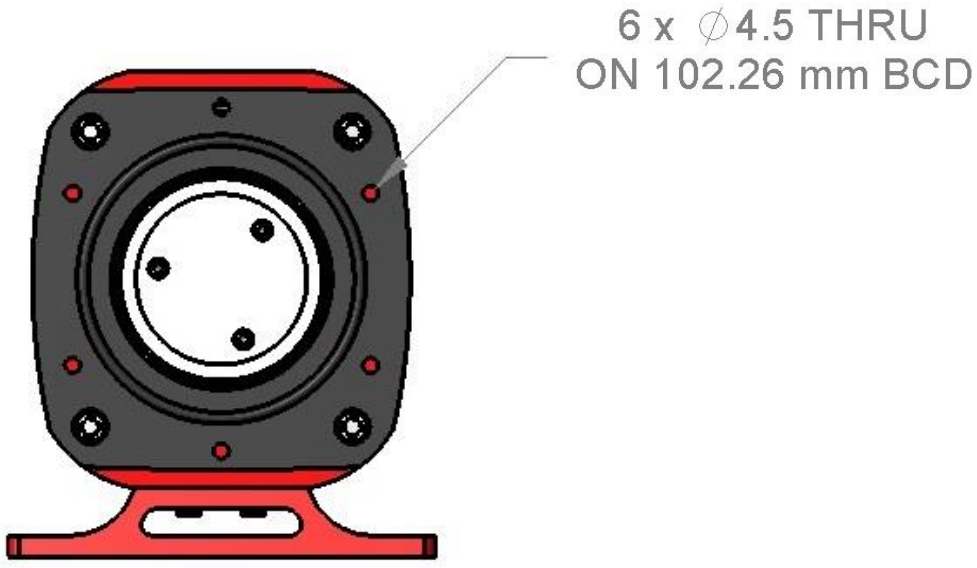
4.9 Pump Mounting Details – Feet Bolt Pattern



4.10 Pump Cylinder Mounting Face Bolt Pattern



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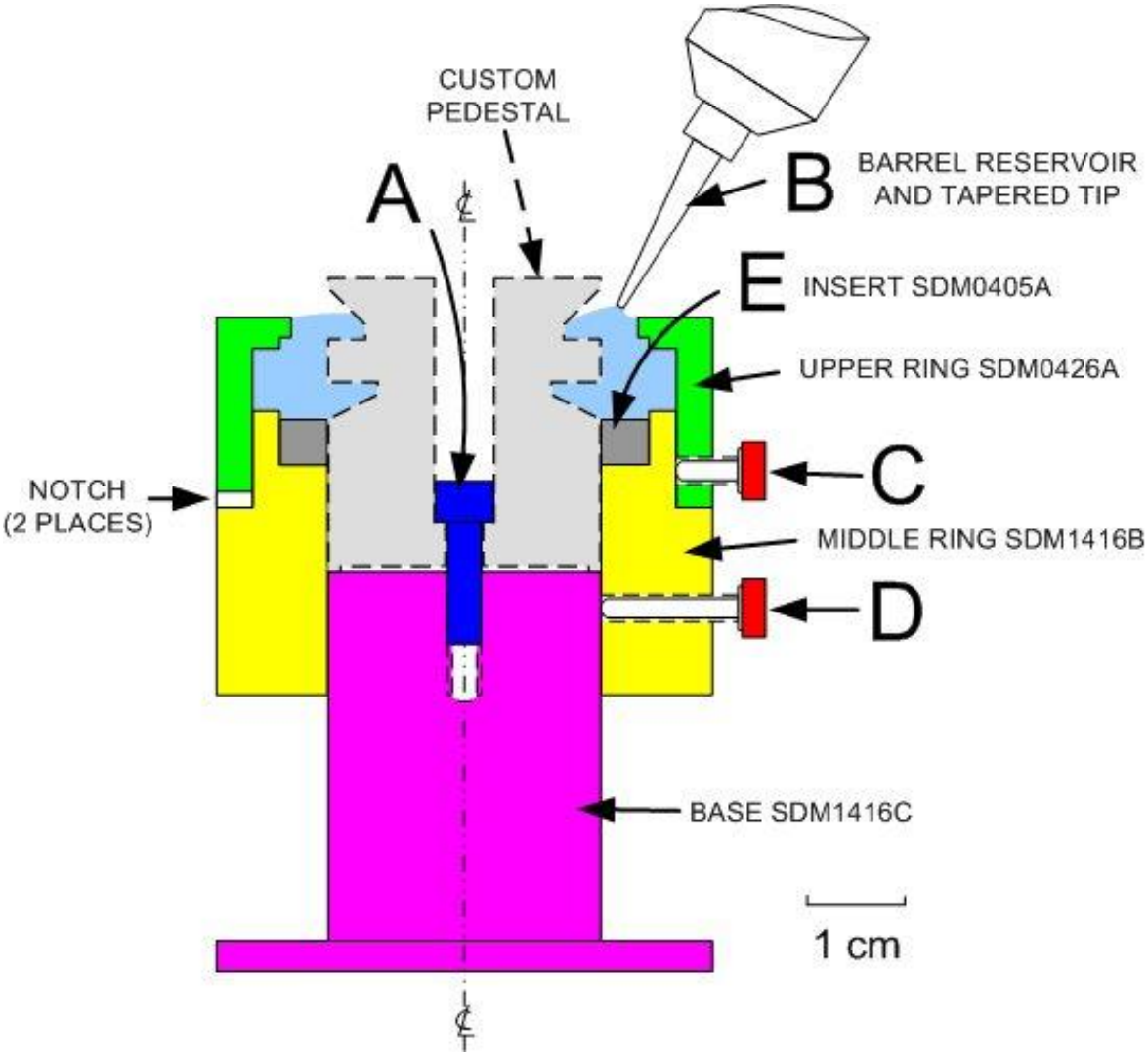
5. Valve Mounting and Molding Procedure

5.1 Rubber Ring Moulding Considerations

Silicone rubber rings are recommended for mounting heart valves or other test specimens. The rubber rings provide a fluid seal between the device under test and the test system flanges.



Since each size and type of test device differs in geometry, a unique rubber ring is required in order to match and seal the appropriate boundaries on the device. The rubber ring should not interfere with the action of the device being tested. The considerations discussed here apply to the design of rubber rings for mounting mechanical or tissue valves, with or without sewing rings.

Molding jig components for valves are shown coloured in the following illustration. The jig components can be separated and adjusted by loosening bolt A and thumb screws C & D.
The custom pedestal (Light GREY) is attached to the base (PURPLE) with a bolt (A). The pedestal can be the actual valve or a replica of the outer dimensions to which the rubber ring seals. It can be made of Delrin™ which has good mold release ability. When a valve or valve

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component is used as a mold, a thin layer of Vaseline coating on the component may facilitate mold release after vulcanization. Vaseline can be washed off using soap and water after the rubber ring cures. In general, to help minimize flow turbulence, a tapered transition should be made between the outside diameter of the rod and the inside diameter of the device inlet orifice.

For tissue valves, it is likely that the tissue will be damaged in the molding process and should therefore be cut away before molding. A tissue-less valve stent, having all component parts except issue, can facilitate molding of complex shapes. If porous fabric materials are to interface with the silicone rubber, a thin layer of mold release (e.g. Vaseline or wax) should be applied before molding. Experimental devices, (e.g., VSI, BC bi-leaflet tissue valve) can use a plastic rather than a fabric cuff. This simplifies the design of the proto-type valve and fabrication of both the mounting and rubber rings.

A molding kit is supplied to assist in making rubber rings. The standard kit consists of the following parts:

- 1 each, molding jig components
- 1.8 pounds, GI-1100 Silicone rubber (Silicones, Inc.)
- 1 each, dispensing syringe (enlarged end port), 35 ml
- 0.2 pounds, GI-1100 Activator (Silicones, Inc.)
- 1 each, dispensing syringe, 1 ml
- 1 each, standard dispensing syringe, 35 ml
- 1 each, gage tapered tip, #5116TT-B (EFD Inc.)
- 100 ml, waxed paper mixing cup and wooden spatula
- 1 ml, mold release grease (Vaseline) in syringe

5.2 GI-1100 Silicone Rubber

This rubber is made by Silicones, Inc., P.O. Box 363, 211 Woodbine Street, High Point, NC, 27260, USA. Ph (919) 886-5018, Fax: (919) 886-7122. It has extremely high tear strength and is a, highly elastic, two-component, tin catalyzed RTV (Room Temperature Vulcanizing) silicone rubber.
Specifications include:

- Hardness, Shore 22 □4
- Tensile Strength (AStm D412) 525 □25 psi
- Elongation (AStm D412) 400 □25%
- Tear, Die B (AStm D624) 140 □15 ppi
- Shrinkage 0.1 %
- Specific Gravity 1.07

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5.3 Ring Moulding Procedure

1. Insert **E (dark GREY)** is removable. This ring should remain in situ when making rubber rings intended for use in the mitral site of the ViVitro Labs Model Heart and load systems. The Insert E (Dark GREY) should be removed when making rubber rings for use in the Leakage Tester and the HiCycle test systems. This ring may also find use as a filler in the aortic valve mounting site when thin rubber rings intended for use in the mitral position are to be used in the aortic valve mounting site.
2. With the middle ring **(YELLOW)** over the base **(PURPLE)**, attach the device or replica **(light GREY)** so that it is centered and square to the base rod **(PURPLE)**. The method of attachment will depend on the device.
3. Fill the assembly with silicone rubber (GI-1100 recommended) which has been de-aerated as described later. Prior to complete jig assembly and filling with rubber, it is recommended that rubber first be applied to deep undercut regions using the barrel reservoir and tapered tip (B). This avoids entrapping air in those regions where the flow of the viscous rubber can be problematic.
4. Place the upper ring (**GREEN**) over the middle ring **(YELLOW)** and lock with thumbscrew (C). Adjust the locked assembly (**GREEN** + **YELLOW**) on the base **(PURPLE)** so that when rubber fills the cavity, the top level of rubber is even with the top edge of the upper ring **(GREEN)** and about 2-3 mm of rubber covers the top ridge of the pedestal or device **(TAN)**. This top covering layer of rubber should be sufficient to secure the test device during forward flow conditions.
5. When the rubber is cured, dismantle the molding jig and remove the rubber ring. Remove the upper ring **(GREEN)** from the middle ring **(YELLOW)**. Two notches on the upper ring **(GREEN)** allow insertion of a blade type screw driver which can help pry the two rings apart.
6. Push the middle ring **(YELLOW)** down over the base **(PURPLE)**.
7. Peel and stretch the rubber ring off the device **(light GREY)**.
8. The rubber ring should be trimmed of any excess rubber using small scissors. Trimming should be done carefully to prevent compromising the seal of the ring.

5.4 Preparation of RTV silicon rubber

Optional accessories recommended for molding silicone rubber are given below. These items may be obtained from the indicated suppliers.

- 35 ml/stroke, hand-operated vacuum pump, capable of generating up to 25 inches of mercury vacuum, # P-79301-20 (Cole-Parmer Inc.)
- 365 ml, Plexiglas vacuum chamber, ¼ inch I.D. tubing, VC9991
- air-powered dispenser controller, gas pressure input 80-100 PSIG; gas pressure output 0-100 PSIG, #800V (EFD Inc.)
- 30 ml Barrel reservoir/pistons, #5112CP-B (EFD Inc.)

NOTE: A standard, hand operated, 35 ml syringe may also be used rather than an air powered dispenser and barrel reservoir/piston combination, however, considerable force of hand is necessary to inject the rubber mixture. The tapered tip (e.g. #5116TT-B) should be attached to the syringe before dispensing the rubber)

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5.5 Mixing

For making one rubber ring, about 15 ml volume of GI-1100 RTV silicone base is required. This volume is best measured and dispensed from the silicone base container using a modified 35 ml syringe which has a large hole in the syringe end. Proceed as follows:

1. Push syringe piston to the end of its travel
2. Insert syringe end into silicone base to a depth of about 2 cm and slowly retract syringe piston to withdraw 15 ml volume. Wipe off excess silicone base from syringe end.
3. Dispense the syringe contents into the mixing cup.
4. Using a 3 ml syringe, dispense 1.5 ml of activator into the cup.
5. Thoroughly stir the mixture with the wooden spatula. Be sure to scrape the sides and bottom of the cup to ensure the correct ratio of base to activator (10:1). Stir slowly until a uniform light blue colour without streaks is achieved.

5.6 De-aerating mixture

Immediately after mixing, de-aeration of the mixed rubber to remove entrapped air is always recommended. Removing air bubbles helps assure the rubber rings sealing ability and provides uniformly dense rings. Proceed as follows:

1. Place the mixing cup with the mixed silicone rubber into a small volume vacuum chamber (e.g. part # VC9991, 365 ml chamber volume).
2. Attach a hand operated vacuum pump to the vacuum chamber using a ¼ inch I.D. hose (e.g. # P 79301-20).
3. Evacuate to about 24 inches of mercury vacuum. The mixture will expand towards the top edge of the cup and then begin to collapse as the entrapped bubbles burst in the low pressure. The mixture should eventually collapse back to its original volume. The bursting of the bubbles can take several minutes however this time can be reduced by providing multiple evacuation/release cycles during the de-aeration process. During this cycling process, the 24 inches of mercury vacuum condition should be maintained for about 2 minutes. Using this method, the total de-aeration time should be about 10 minutes.
4. Release vacuum and remove the cup from the chamber
5. Pour and spatula the mixture into a 30 ml barrel (e.g. #5112CP-B) and insert piston.
6. Expel barrel air as much as possible.
7. Attach tapered tip (e.g. #5116TT-B) to loaded barrel.
8. Attach the barrel adapter assembly to an air pressure source (e.g. #800V) and set pressure to 40 psig.
9. Slowly inject the mixed silicone rubber into the cavity being careful to avoid the formation of air bubbles or entrapment of air. Allow the rubber to flow around the cavity. The time to fill the cavity with rubber should be about 10 minutes.

Allow the rubber to cure (vulcanize) for 16 to 18 hours at 25 C. Lower temperatures and/or low humidity will cause the cure-time to lengthen; conversely, higher temperatures and/or high humidity will cause the cure-time to shorten.

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6. Pulse Duplicator System Assembly

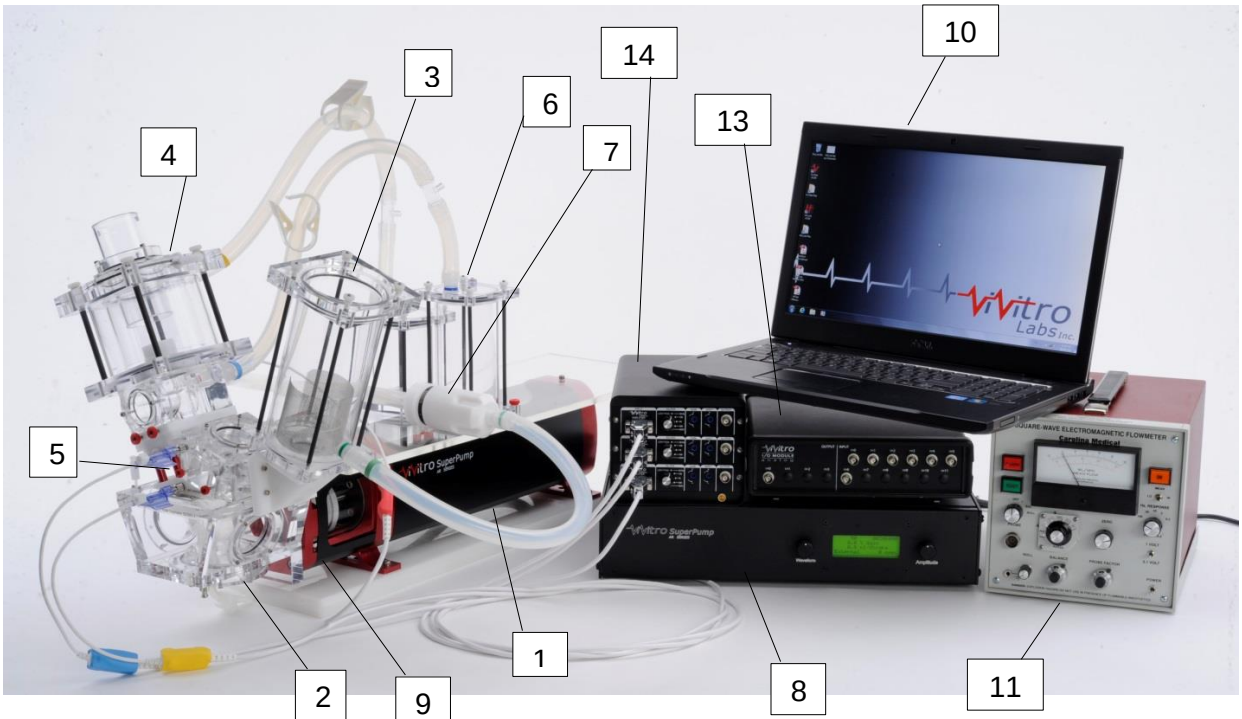
The Pulse Duplicator System has several steps to assemble the main mechanical sub-assemblies, and a few steps for the electrical components installation/configuration.

NOTE: It is critical for the assembly steps to be followed in order to assure successful operation.

6.1 Unpacking – List of Components

Inspect the shipping container for external damage. Retain the shipping carton and packing materials for possible re-shipment. Inspect the system components for damage. If there is evidence of damage contact the carrier, initiate a damage claim, and inform ViVitro Labs by calling 1-250-940-2420 or toll free at 1-877-588-3531. The following assemblies should be identified on unpacking:

1. SuperPump
2. Ventricle Chamber
3. Atrium Chamber
4. Aortic Chamber
5. Aortic Standoff and and Substitute Probe
6. Compliance Chambers and Tubing
7. Peripheral resistance controller
8. SuperPump Controller
9. Viscoelastic Impedance Adaptor (VIA)
10. Computer
11. Flow Meter and Connector Cables
12. Flow Probe (Not Shown)
13. I/O Module and Connector Cables
14. Ampack, Pressure Transducers, and Connector Cables
15. Molding and Accessories Kit (Not Shown)



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Optional hardware:

- Heat Exchanger and Heat Bath
- Large Compliance Chamber
- LVOT accessories
- Transfemoral and Transapical Access Device
- Digital Manometer

The following steps detail procedures for assembling the main components outlined above in the order that they should be installed for a complete unit.

6.2 SuperPump

The SuperPump arrives pre-assembled with the following parts:

- Base Plate
- Linear Actuator Cylinder
- Standard Pump Head

WARNING: It is recommended that the SuperPump be positioned with its longest dimension horizontal. Should the pump be vertically mounted with the pump head above the motor the possibility of liquid contamination of the mechanical and electrical parts is likely to occur.

NOTE: The pump head is made from an electrical non-conductor. In applications where an electromagnetic flow transducer is used this will isolate the path to ground for the transducer from the ground path for the motor. This should improve the signal to noise ratio of the flow signal.

Locate the SuperPump on a fixed structure, i.e. a table or frame suitable to accommodate the weight and dimensions of the entire system once put together. The pump head of the SuperPump should extend over the edge of the bench to provide clearance for the ventricle drain assembly.

6.3 Attaching the VIA and the Ventricle Chamber

Fasten the VIA directly to the SuperPump using 3x #8-32 x 1” screws and flat washers. Tighten the fasteners evenly and ensure the fasteners are not overtightened.

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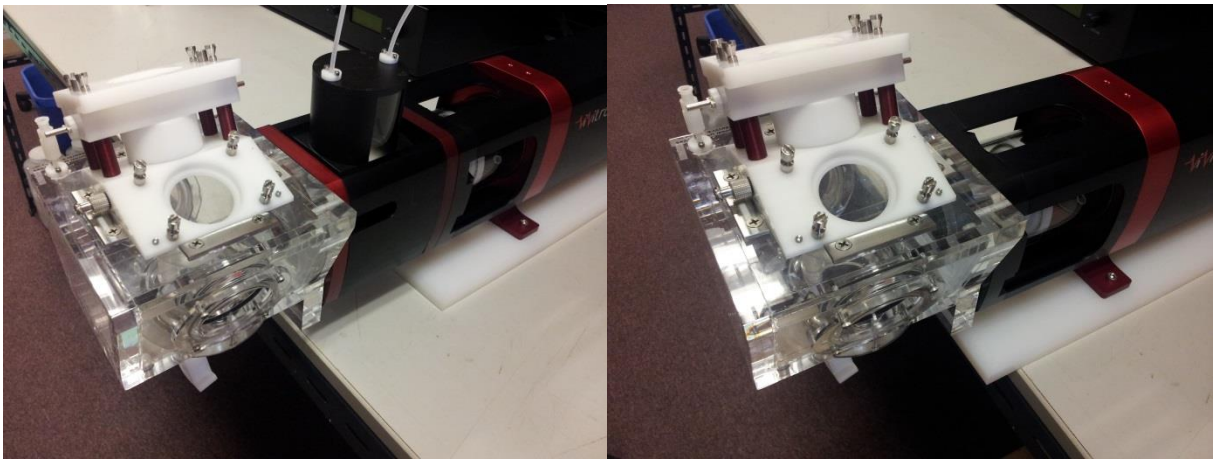
6.4 Attaching the Ventricle Chamber

NOTE: The three screws should be tightened evenly and NOT BE OVER-TIGHTENED. Be careful that the bolt entering the blind tapped hole in the Ventricle Chamber does not bottom out when tightened as this will damage the acrylic material of the chamber.

Fasten the Ventricle Chamber to the VIA using 3x #8-32 x 1" screws and flat washers. Tighten fasteners evenly and be extremely careful not to overtighten an damage the acrylic. If no VIA is being used with the system.



6.5 Filling the Ventricle Chamber, VIA (if installed) and Pump Head

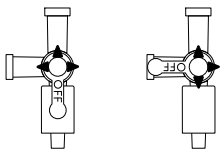
REQUIREMENTS: 0.8 to 1.5 liters of de-aerated fluid. It is further recommended to use distilled water with biocide to reduce bioburden. An appropriate biocide can be determined by a reputable supplier.

NOTE: It is recommended that the ventricle chamber be filled with de-aerated fluid to minimize the formation of small air bubbles which may reduce visibility and introduce artifact in echo studies.

NOTE: Ensure the SuperPump is set at the "home" position in the centre of its range movement before filling, therefore creating minimal expansion or collapse of the ventricle membrane during operation.

1. Fill the ventricle chamber with fluid (0.8 liters or 1.5 liters with VIA) via the stopcock. This will fill the pump head, ventricle chamber and VIA (if installed). Fill until the ventricle membrane is slightly compressed. Be sure not to overfill the ventricle chamber which will collapse the ventricle membrane.

CLOSE **OPEN**



Relevant stopcock positions

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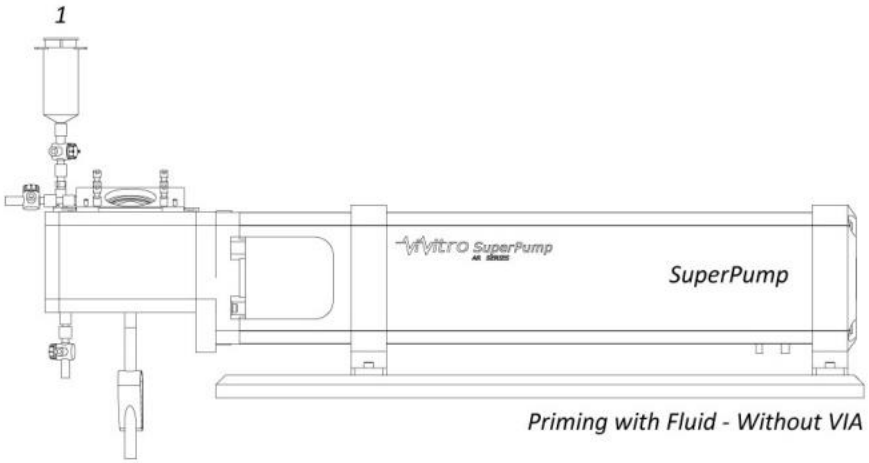
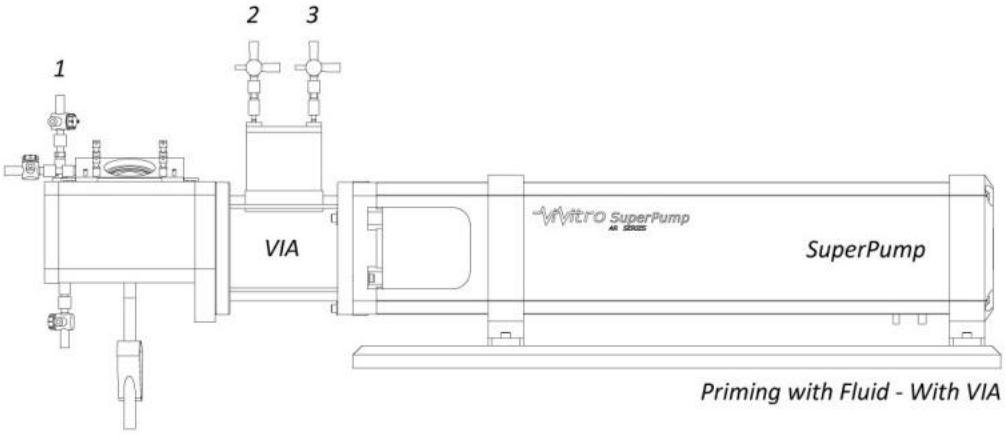


Priming with Fluid - Without VIA



Priming with Fluid - With VIA

2. Close the 2 stopcocks on the VIA (if installed).
3. Remove air by syringe via the stopcock on the ventricle chamber. This involves adding a predetermined amount of fluid and removing an equal amount of air, so that the ventricular membrane is not over or under expanded.

Debubble the ventricle chamber and pump head by:

- attaching a syringe to the stopcock on the ventricle chamber;
- open the stopcock on the ventricle chamber;
- if VIA installed, close the two stopcocks on the VIA;
- elevate the whole system at the ventricle chamber by approximately 30º;
- debubble/remove the air in the chamber;
- return to horizontal position when debubbling is completed.

Debubble the VIA (if installed) by attaching a syringe to the stopcock closest to the ventricle chamber on the VIA, then:

- open the stopcock on the VIA;
- close the remaining stopcocks: one on the ventricle chamber and the other on the VIA;
- elevate the whole system at the ventricle chamber by approximately 30º;

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- debubble/remove the air in the chamber;
- return to horizontal position when debubbling is completed.

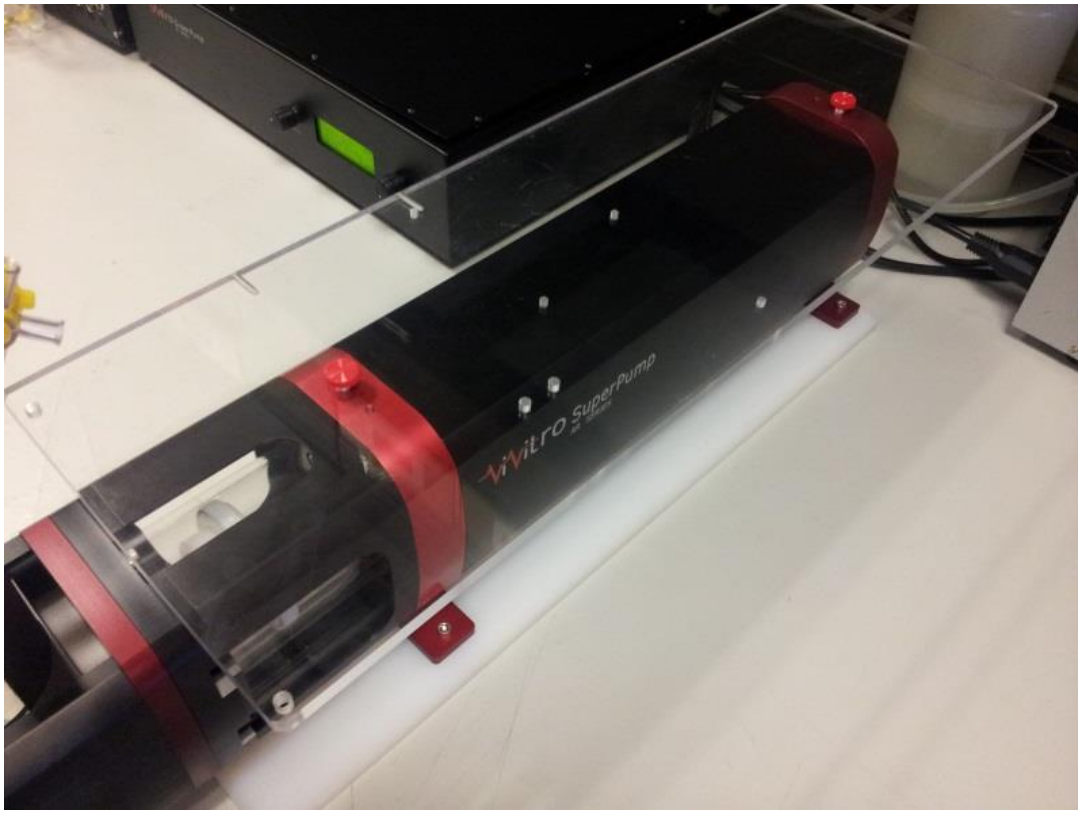
Adjust the fluid volume of the ventricle chamber so that the ventricle membrane is in a relaxed state by:

- attaching a syringe to the stopcock on the ventricle chamber;
- open the stopcock;
- closing the stopcocks on the VIA (if installed);
- add fluid to the ventricle chamber ensuring the ventricle membrane is in a relaxed state.

NOTE: The fluid in the ventricle chamber can remain but should be replaced periodically (<2 months). If the system is used infrequently, drain fluid from the chamber and allow it to dry.

6.6 Accessories Table Installation

An accessories table has been supplied in the model left heart crate to accommodate the peripheral resistance and supplementary compliances. Mount the accessories table to the provided flanges on the SuperPump using the two #10-32 x 1" red thumb screws provided. The two threaded holes in the edge of the table for mounting the optional heat exchanger should be at the front left of the SuperPump.



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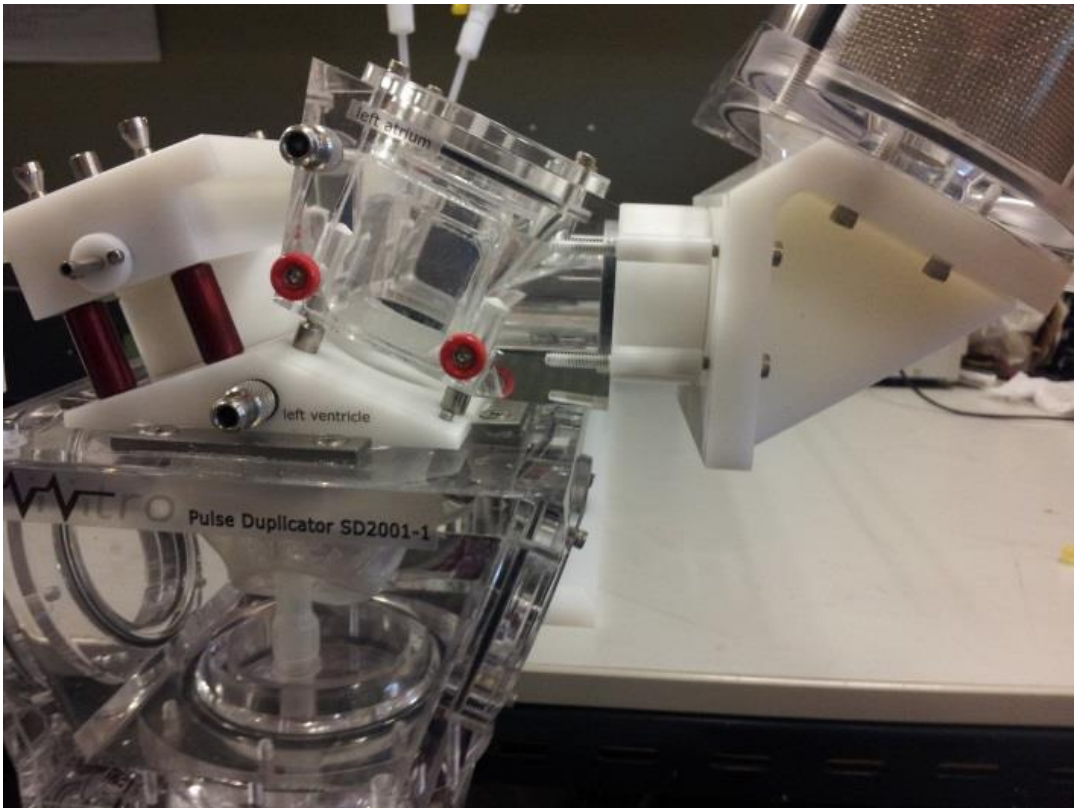
6.7 Atrium Chamber Installation

NOTE: If you wish to install a mitral flow meter electromagnetic probe you may wish to do this prior to installing the Atrium Chamber (see 'Installing the mitral flow probe').

1. Mount the atrium base plate assembly on to the four long stainless steel screw posts situated on the mitral valve side of the mounting block.
2. Tighten the red thumb screws on the assembly to provide a seal around the valve ring.



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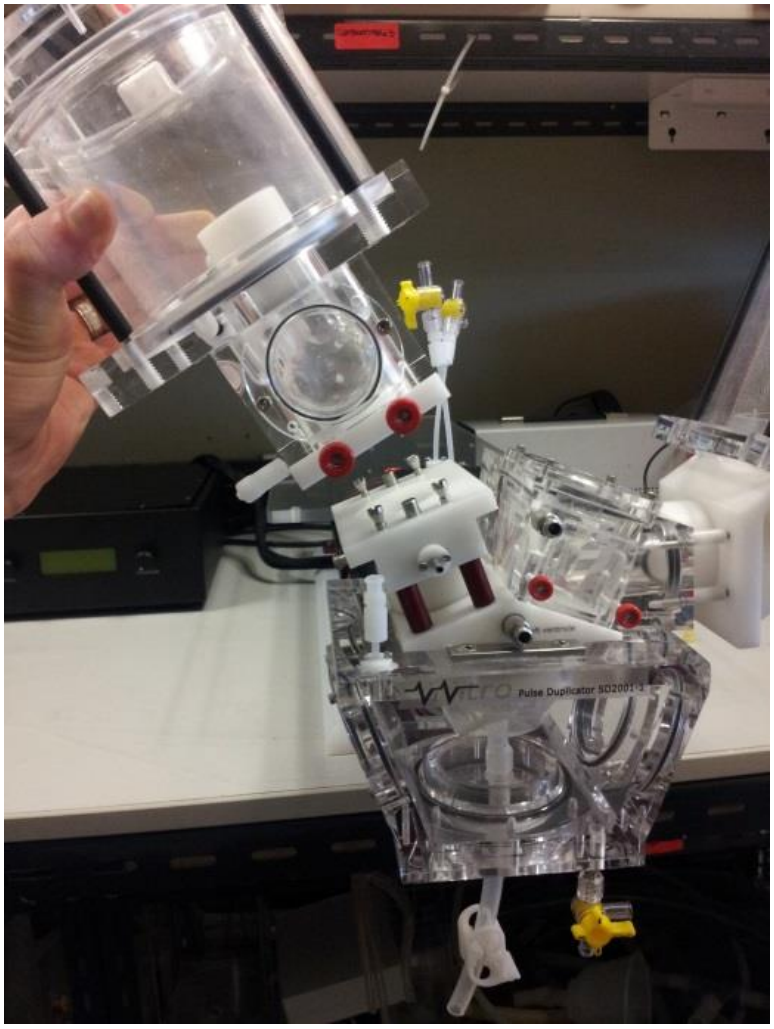
6.8 Aortic Assembly Installation

NOTE: If you wish to install an aortic flow meter electromagnetic probe you may wish to do this prior to installing the Aortic Chamber (see 'Installing the aortic flow probe').

Mount the aortic chamber on the aortic standoff over the ventricular outflow tract flange (over the aortic valve). Tighten the red headed thumbscrews.



NOTE: When positioned correctly, the side of the aortic assembly with the three ports colour coded **RED, YELLOW,** and **BLUE** should be facing towards the accessories table.

NOTE: The aorta viewing tube and window assembly is adjustable and is locked into position using three bolts. The viewing tube window should never be closer than 20 mm from the end of the aorta outlet tube.

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6.9 Flow Probe(s) Initial Operational Check

Components:

- Flow Meter
- Flow Probe
- PHONO to BNC Cable

Before installing the flow probe(s), an initial operation check of the flow measurement system should be performed as follows.

NOTE: This should be done for each flow probe and its accompanying flow meter.

1. Check that the voltage switch on the back panel of the unit is set for your mains supply (115 or 230 VAC). If it must be switched, ensure the correct fuses are installed in the meter as per the instructions on the rear of the unit. If it is necessary to change the power plug style then the following conductor colours apply:

black=phase, white=neutral

2. Check POWER OFF (down) and PROBE switch OFF
3. Plug the power cord into the mains supply.

NOTE: The Flow meter must be properly grounded to the probe site.

4. Connect PULS output on the back panel to the INPUT CH. 4 on the I/O Module.
5. Place the flow probe and ground lead into a non-metallic container filled with a conductive fluid such as saline. (It does not function in air or pure water.)
6. Insert the ground lead cable into the flow meter ground jack on the front panel of the meter.
7. Connect the 8-pin probe plug to the flow meter jack with the correct registration as indicated by the missing pin.
8. Set the MEAN switch to HI.
9. Set the outer dial on the RANGE switch to give a PFX (probe factor multiplier) of 1.
10. The Hz RESPONSE and PROBE FACTOR controls should be left as set during ViVitro Labs Inc calibration as shown on the calibration sheet.
11. Turn the POWER switch on.
12. A switch transient may cause the **ALARM** indicator to illuminate. Depress the **ALARM** indicator to reset the meter. Allow ten minutes for the instrument to stabilize.

NOTE: The green **READY** light should remain on when the meter is measuring flow.

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13. **With the PROBE switch set to OFF** adjust the ZERO control to give a display meter reading of zero. The ZERO control is a 15-turn potentiometer and normal setting would be approximately mid-position, 7 ½ turns clockwise from the extreme anti-clockwise rotation.

NOTE: ZERO control is functional with or without the flow probe attached and the “no-signal” level of the back panel MEAN and PULS outputs are set by the ZERO control. The “with-signal” level should be approximately the same as the “no-signal” level under static flow condition.

14. **With the PROBE switch set to NULL** adjust the NULL control until the display meter indicates a MINIMUM reading.
15. **With the PROBE switch set to BALANCE** rotate the BALANCE control so that the meter reads approximately zero. This position should be close to that set by ViVitro Labs Inc during pre-shipment calibration.
16. **With the PROBE switch set to either + or -** (Normal placement of the probe, dictates flow is in the “+” direction) If necessary, use the ZERO control until the meter shows zero.

NOTE: If the meter is off scale, check for improper grounding or air bubbles in the probe lumen which can be removed using a cotton swab. Alternate + and – while adjusting Balance for least difference.

17. Move the probe so that an oscillatory flow of saline moves through the probe lumen (caused by moving the hand or implement in the fluid manually). The display meter should show an oscillatory signal above and below zero. The back panel PULS output should also follow this signal. This may be verified on a flow monitor or via the ViViTest software.

Turn the PROBE switch OFF when flow is not being monitored.
Successful completion of the above steps indicates a functioning flow meter system and mounting of the flow probe into the Pulse Duplicator System can proceed. Once mounted in Pulse Duplicator System, step 5 and 17 are not applicable.

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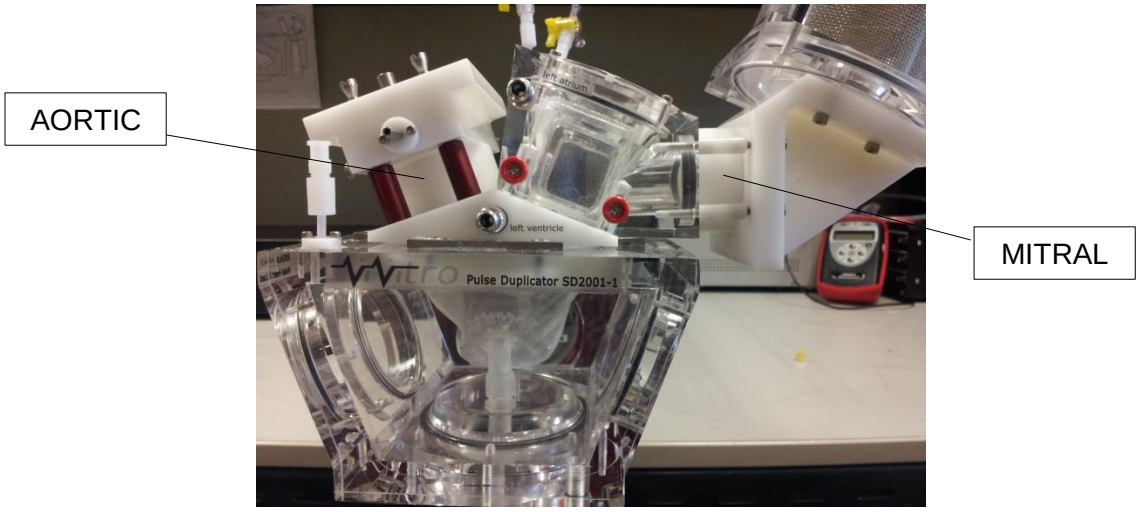
6.10 Installation of Flow Probes

There are two positions on the Pulse Duplicator System where electromagnetic probes may be installed in the place of substitute probes:

- in the aortic site and
- the mitral site



When a single probe/flow meter is used, the displacement of the pump is differentiated over time to obtain the total flow into the system, and then the measured flow from the site with the sensor is subtracted from this number to obtain the flow in the site without a sensor. In every case, a ground connection between the flow meter and the probe site is necessary. SYNCH on the back panel of the flow meter is used only when two flow meters are connected.

6.11 Installing the Aortic Flow Probe

The aortic probe is located between the ventricle chamber and the aortic chamber on the aortic standoff.

1. Remove the aortic assembly (loosen thumb screws and lift off tapered screw posts.
2. Unscrew the four tapered screw posts to remove the white mounting on the aortic standoff. This will reveal a further 4 screws to remove the remaining structure. Remove the four screws to unclamp the substitute flow transducer (white Delrin spacer).
3. Remove substitute flow probe.
4. Insert electromagnetic flow probe using the supplied flow channel alignment jig to assist in centering the flow probe between the aortic housing clamp, ventricular outflow tract and valve mounting base. Take care to align the flats on the alignment jig with the probe electrodes to prevent marring the electrodes.
5. Replace and evenly tighten the four bolts. Do not use excessive torque.
6. Replace and adjust the four tapered studs so that when the thumbscrews are being tightened onto the tapered surface of these studs, the mating flanges pull together and provide a seal around the silicone ring.

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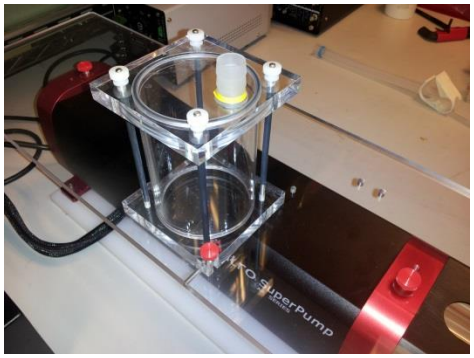
6.12 Installing the Mitral Flow Probe

The mitral probe is located between the ventricle chamber and the atrium chamber.

1. Remove the left atrium assembly (loosen the four thumbscrews and lift).
2. Loosen the four bolts securing the mitral flow channel to the atrium chamber.
3. Remove white Delrin spacer.
4. Insert electromagnetic flow probe. Carefully align probe to minimize turbulence.
5. Tighten evenly the four bolts. **Do not use excessive torque.**

6.13 Aortic Compliance Chamber Installation

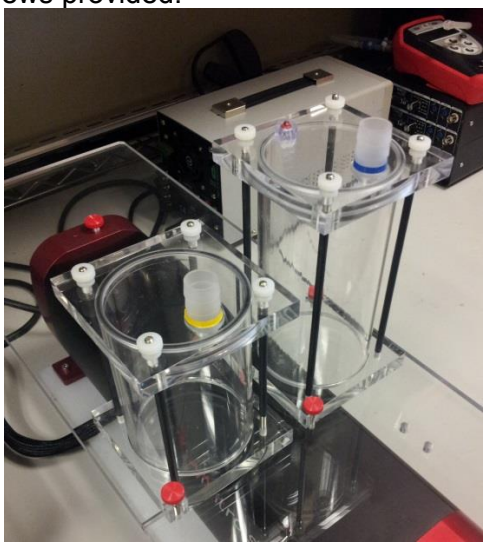
1. Mount the Aortic Compliance Chamber on to the Accessories Table using two red #8-32 x ¾" thumb screws provided.



2. Connect the silicone tube from the top of the compliance chamber to the Aortic Chamber at the marked YELLOW outlets/inlets.

6.14 Aortic Room Compliance Chamber

1. Mount the Aortic Root Compliance Chamber on to the Accessories Table using two #8-32 x ¾" red thumb screws provided.



2. Connect the silicone tube from the top of the compliance chamber to the Aortic Chamber at the marked BLUE outlets/inlets.

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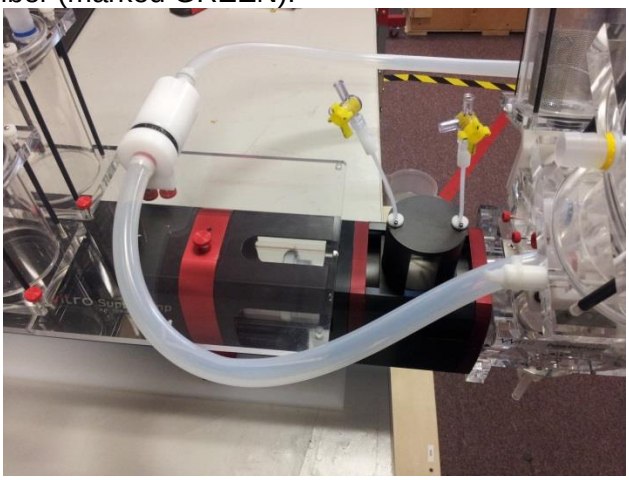
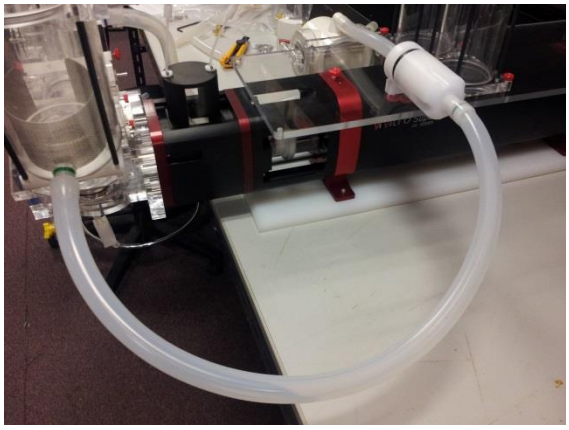
6.15 Peripheral Resistance Controller

1. Mount the Peripheral Resistance Controller on the Accessories Table using the two #10-32 x 1 ¼" screws provided.
2. Connect the silicone tube from the outlet (marked GREEN) of the controller to the inlet of the Atrium Chamber (marked GREEN).



Connections with no Heat Exchanger

3. If no heat exchanger is being used, connect the silicone tube from the RED outlet of the Aortic Chamber to the RED inlet of the controller. If using a heat exchanger, proceed to Section 6.16.



Connections with a Heat Exchanger

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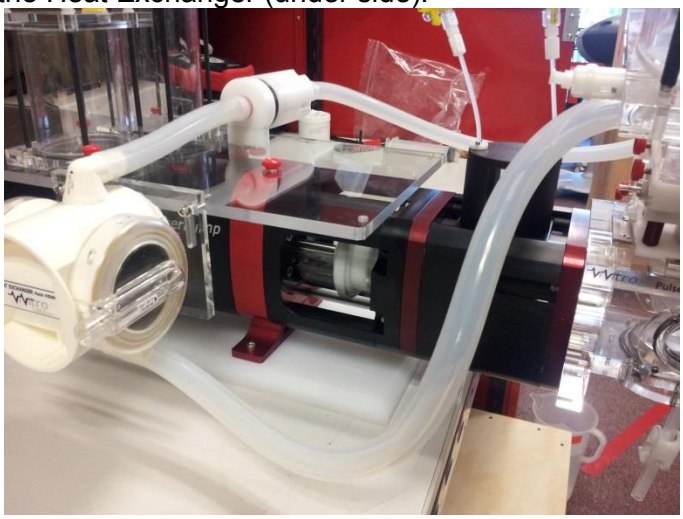
6.16 Heat Exchanger System Installation (Optional)

Components:

- Heat Exchanger with Mounting Flange
- Heat Bath

1. Mount the Heat Exchanger to the side of the Accessories Table (where screw holes are located).
2. Connect the silicone tube from the 'Test Fluid Outlet' (top) of the Heat Exchanger to the Peripheral Resistance Controller.
3. Connect the silicone tube from the outlet of the Aortic Chamber marked RED to the 'Test Fluid Inlet' on the Heat Exchanger (under side).



4. Connect the silicone tube from the right inlet on the Heat Exchanger to the outlet on the Heat Bath.
5. Connect the silicone tube from the left outlet on the Heat Exchanger to the inlet on the Heat Bath.
6. Fill the Heat Bath with fluid.
   NOTE: It is advised to add an algaecide to prevent algae growth.
7. Turn the Heat Bath on and set to desired temperature (i.e. 37.0ºC).

6.17 Silicon Hose Installation

Confirm that the four colour coded silicone rubber hoses are connected correctly between colour coded ports:

1. YELLOW: from the port on the top of the aortic assembly to the smaller of the two compliance bottles. This provides characteristic compliance supplementary to that in the aortic assembly.
2. BLUE: from the port to the air volume surrounding the aortic root to the larger of the two compliance bottles. This provides supplementary aortic root compliance.
3. RED: from the outlet port in the aortic root enclosure to the inlet of the peripheral resistance (or the heat exchanger inflow port.)
4. GREEN: from the outlet of the peripheral resistance to the inlet of the left atrium assembly

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6.18 Electrical Connections

The 6 electrical components used to control and monitor the mechanical components of the Pulse Duplicator System are:

1. SuperPump
2. SuperPump Amplifier
3. Flow Meter
4. AmPack
5. Computer
6. I/O Module

See Page 42 for an annotated image of the components.

STOP: CHECK YOUR COUNTRY'S POWER SUPPLY AMPERAGE AND VOLTAGE PRIOR TO CONNECTING INTO A POWER SUPPLY

ViVitro Labs Inc. will endeavor to have your system's components adapted to meet your country's power supply, however, as an extra precaution, it is advised that all equipment designated to be connected to a power supply be checked prior to use.

1. Check that the voltage setting on the back panel of the Flow meter and AmPack are set for your supply voltage BEFORE connecting to the power supply. Ensure that the operating voltage of the Heat Bath is correct for your region before connecting to the power supply.
2. Where the voltage setting is incorrect adjust the setting manually according to the hardware's instructions.
3. Where it is necessary to adapt the power supply plugs the following line cord colours should be observed.

black=phase, white=neutral, green=ground.

The diagram on the following page shows a schematic detailing the electrical connections that need to be made to make the system operational.

The following table details these connections:

Diagram Colour Reference		Hardware From	Label	Hardware To	Label
	Red	AM Pack	Bottom Left	Aortic Chamber	Aortic Transducer
	Dark Red	AM Pack	Bottom Right	I/O Module	CH 0 (INPUT)
	Yellow	AM Pack	Middle Left	Ventricle Chamber	Ventricular Transducer
	Orange	AM Pack	Middle Right	I/O Module	CH 1 (INPUT)
	Sky Blue	AM Pack	Top Left	Atrium Chamber	Atrial Transducer
	Dark Blue	AM Pack	Top Right	I/O Module	CH 2 (INPUT)
	Green	SuperPump Amplifier	DL/DT OUTPUT	I/O Module	CH 3 (INPUT)
	Navy Blue	Flow Meter	PULSE	I/O Module	CH 4 (INPUT)
	Green	Flow Meter	Probe Cable	Aortic or Mitral Flow Probe	Flow Probe location
	Black	Flow Meter	Ground	Aortic or Mitral Standoff	Grounding Pin
	Grey	SuperPump Amplifier	POSITION OUTPUT	I/O Module	CH 5
	Brown	SuperPump Amplifier	DRIVE INPUT	I/O Module	CH 0 (OUTPUT)
	Black	SuperPump Amplifier	LINEAR ACTUATOR	SuperPump	Motor
	Green	I/O Module	USB	Computer	USB

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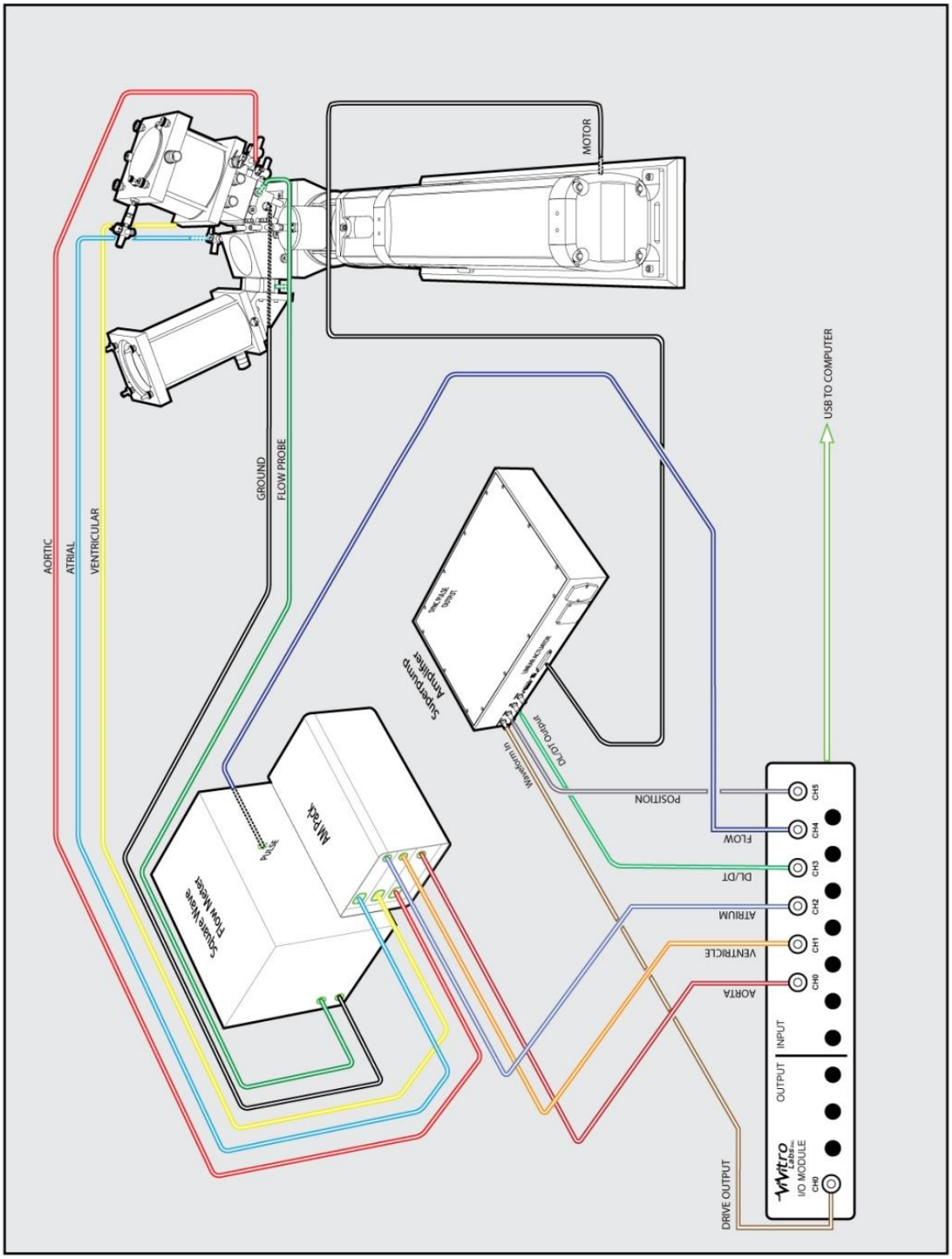
6.19 Electrical Connection Diagram



Electrical connections for the Pulse Duplicator System

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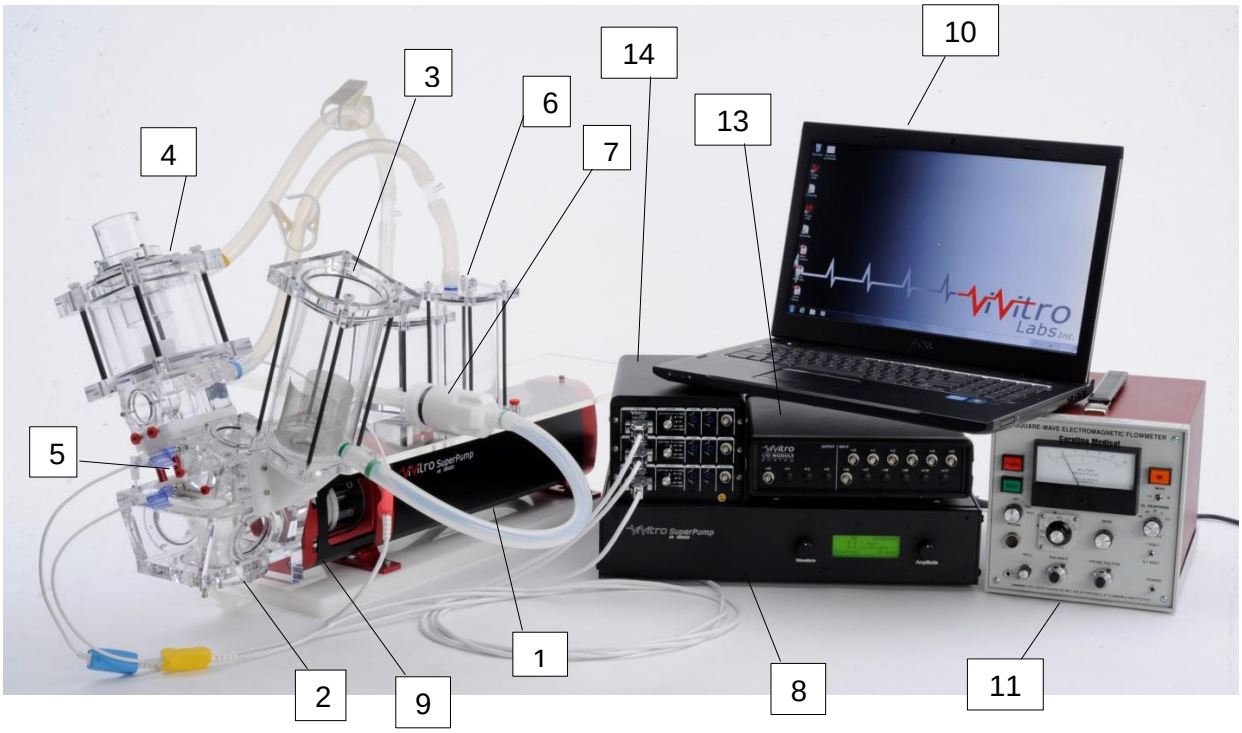
7. Testing Set-up Procedures

The following sections describe the steps required to prepare an assembled system for testing.

7.1 Initial Assembly Procedure

Refer to Section 6 to ensure the systems initial assembly is complete before continuing. Going forward it is assumed that all the hardware and electrical components have been connected. The following sections provide the user with the procedures for set-up of the Pulse Duplicator System for testing. These procedures provide operation for ideal physiological conditions known to ViVitro Labs. The diagram below provides an illustration of the fundamental set-up, and operational depiction.

1. SuperPump
2. Ventricle Chamber
3. Atrium Chamber
4. Aortic Chamber
5. Aortic Standoff and and Substitute Probe
6. Compliance Chambers and Tubing
7. Peripheral resistance controller
8. SuperPump Controller
9. Viscoelastic Impedance Adaptor (VIA)
10. Computer
11. Flow Meter and Connector Cables
12. Flow Probe (Not Shown)
13. I/O Module and Connector Cables
14. Ampack, Pressure Transducers, and Connector Cables



The Standard Pulse Duplicator System.
A complete list of components for each assembly can be found within the Appendices.

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7.2 Installation of Valves

NOTE: A silicon rubber ring around the valve ring or sewing ring should be used for mounting valves in the Pulse Duplicator System. This ring also provides the seal between the atrium chamber and the mitral valve on the ventricle chamber mounting block and between the aortic valve mounting flange and the aortic standoff. The soft rubber also helps simulate compliant tissue.

The valves in their rubber rings can be inserted into the valve mounting flanges. The mitral valve corresponds to the site with the longer tapered studs in the valve mounting block. The aortic valve mounts in the aortic outflow tract downstream of the aortic flow transducer or substitute Delrin ring. Note that the valves in their rings can be rotated around their stream wise axis and mounted in different orientations. The mitral and aortic valves are retained by the atrium and aortic standoff, respectively.

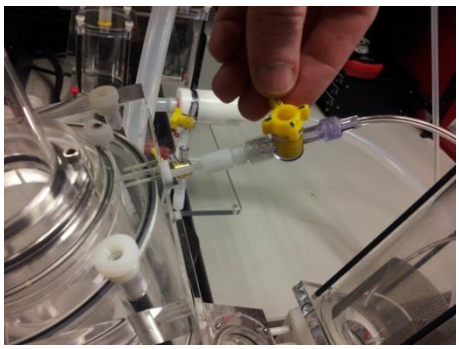
7.3 Priming / Preparing the Chambers

1. Add the test fluid through the atrium chamber, located above the mitral valve
2. The Aortic, Ventricle and Atrium Chambers will fill with fluid. Continue until fluid is near the top of the aortic outflow in the Aortic Chamber.

7.4 Air Bubble Removal

To ensure the data you acquire is accurate, it is important to debubble the chambers prior to running your tests.
Instructions:

1. Install a catheter line to the stopcock at the top of the aortic chamber and place the end of the catheter in the atrium chamber to collect overflow
2. Ensure the lid is fastened to the top of the aortic chamber
3. Check that the peripheral resistance controller is open at least two full turns



4. Via the Amplifier:
    a. Start the SuperPump by slowly turning the Amplitude knob
    b. Adjust the Amplitude setting to a low stroke volume (approx. 30 mL/stroke)
5. Open the stopcock to vent air from the inside of the aortic chamber.

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6. Add fluid to the atrium as necessary to the system to prevent air bubbles from being circulated.
7. Close the stopcock when all air has been displaced by test fluid

NOTE: It may be necessary to increase the pumping rate/stroke volume and reduce the Peripheral Resistance to remove all air bubbles. Monitor aortic pressure and avoid excess pressure in the system.

7.5 Characteristic Compliance and Liquid Level Adjustment

Note: The instructions for Aortic Root Compliance Chamber and Aortic Compliance Chamber fluid levels should be conducted simultaneously.

Aortic Root Compliance

1. With the stroke volume set at a given value (e.g. 70 ml @ 70 beats/minute) the pressure of the air above the fluid surrounding the Aortic Root should be adjusted so that the highest level of fluid in the aortic housing almost reaches, but does not occlude the tube port making connection to the Aortic Root Compliance Chamber.
2. Using a syringe apply or release air as needed to the Aortic Root Compliance Chamber by way of the luer fitting connected to the tube at the compliance chamber.

NOTE: If fluid enters the aortic root tube it will alter the Aortic Pressure waveform. This will be noticed by a dip in the Aortic Pressure waveform after the Ventricle Pressure drops.

Result:

- Approximate air volumes will be:

	Aortic Root Chamber	35 ml
plus	Aortic Root Compliance Chamber (incl. hose)	615 ml
Totaling		640 ml

If desired, this volume and hence Aortic Root Compliance can be reduced by adding water to the Aortic Root Compliance Chamber to reduce the air volume.

Aortic Compliance

The fluid in the Aortic Chamber should be allowed to rise approximately 2.5 cm.

1. Using a syringe apply or release air as needed to the Aortic Compliance Chamber by way of luer fitting connected to the tube at the compliance chamber.

Note: During collection of low cardiac outputs (e.g. 2 LPM C.O.), it may be necessary to clamp the Aortic Compliance Chamber hose to reduce the compliance, see comments in **Pressure Waveform Adjustment** section.

Result:

- Approximate air volumes will be:

	Aortic Chamber	300ml
plus	Aortic Root Compliance Chamber (incl. hose)	350ml
Totaling		650ml

If desired, this volume and hence Aortic Root Compliance can be reduced by adding water to the Aortic Root Compliance Chamber to reduce the air volume.

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7.6 Peripheral Resistance Adjustment

The peripheral resistance can be adjusted to regulate aortic pressure. This will also affect cardiac output.

NOTE: The peripheral resistance adjustment will also influence the pressure throughout the system so it is recommended to make incremental adjustments while monitoring the air level of the compliance chambers and mean aortic pressure. Care should be taken NOT to occlude the Peripheral Resistance or connection tubing completely. This would rapidly cause excess pressure. Adjustment of the Peripheral Resistance may also change the Aortic Root external fluid level. This level should be adjusted as shown in Section 7.5.

1. Rotate the peripheral resistance outer housing clockwise to increase its resistance to flow. The vernier scale can be used as a guide for repeatability.

2. Monitor the mean Aortic Pressure on the ViViTest software while increasing the stroke volume to ensure that you do not over pressurize the system (for concise instructions see ViViTest Software Operating Manual).
3. During normal operation, the peripheral resistance should be adjusted so that this pressure ranges between 80 and 120 mmHg.

7.7 Pressure Waveform Adjustment

The shape of the Aortic and Ventricle Pressure waveforms is influenced by the Aortic Root and Aortic Compliance Chambers. Additionally, the Viscoelastic Impedance Adapter (VIA) may influence these waveforms. In particular, reduction of the Compliance Chamber pressure will increase the ratio of maximum to minimum Aortic Pressure. At low stroke volumes (e.g. 35 ml/stroke ~2 LPM C.O.) elimination of this compliance may give Aortic Pressure wave shapes closer to physiologic ones. Normally, the Aortic Root Compliance Chamber air volume is left at its maximum value of 640 ml.

Instructions:

1. The tubing to the air compliance chambers can be occluded by closing the plastic clamps. These clamps can be closed just prior to reducing pump stroke volume to zero to prevent air bubbles from entering the test fluid flow loop.
2. You may also release air from the Aortic Root Compliance Chamber when slowly turning down the stroke volume to maintain the proper water levels. This helps to stay prepared for continuous collections.

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7.8 Viscoelastic Imedance Adapter (VIA) adjustments

The VIA will increase ventricle compliance and reduce the rate of increase of ventricle pressure during ventricle contraction. The VIA action consists of the resistance element and two air compliance chambers.

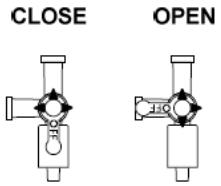
- Vary the volume of air in the output chamber and source chamber to attain the desired pressure waveform.

7.9 Mean Atrial Pressure

The Mean Atrial Pressure value can be adjusted by altering the fluid level in the Atrium Chamber. There should be 10-20 cm of fluid above the mitral valve.

7.10 Transducer Settings

Four ports with stopcocks are provided for the insertion of pressure transducers to measure Aortic, Aortic outflow tract, Ventricle, and Atrial pressures.
Instruction:



- Insert the pressure transducers directly into the stopcocks at the pressure measuring ports.
- Care should be taken to remove air bubbles in the fluid line from pressure transducers by flushing the line using a syringe (distilled water is recommended). Attach the syringe to the outer luer fitting, open inner and outer fitting, and slowly pull fluid through transducer. Repeat until all bubbles are removed and away from transducer sensor.
- Apply atmospheric pressure to the pressure transducers by turning the appropriate stopcocks to the closed position (this allows the transducer to measure air/atmospheric pressure).

NOTE: Excessive tightening of the Luer lock cap should be avoided. Pressure transducers may be damaged by over-pressure. This can occur if a finger occludes the transducer pressure port during transducer flushing. RAPID STOPCOCK CLOSURE may also produce excess pressure. Please contact ViVitro Labs Inc. regarding use of other pressure transducers with the ViVitro Labs Inc. Model AM9991 amplifiers (e.g. Millar catheter tip MPC-500).

NOTE: Atmospheric pressure is useful as a zero pressure reference. The pressure on the transducer sensing element will be atmospheric plus or minus the static head. This includes fluid in the stopcock and in the pressure transducer. If this static head and atmospheric pressure do not change (i.e. orientation of transducer and atmospheric pressure are constant) then the reference pressure will be constant for each transducer.

NOTE: With no flow of test fluid, the pressure transducers will record the static head of pressure on the transducer sensing element. This will differ from the static wall pressure by an amount equivalent to the head level difference between wall pressure measuring point and the sensing element. To compute transvalvular pressure under pulsatile flow conditions, compensation for the static head of pressure should be made. This compensates the pressure transducers to a zero offset for differential measurement, referenced as compensated pressure.

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Experimental Measurement:

- Experimental measurement of the compensation to be applied can be made with the same fluid head in the model above all pressure measuring ports.
- The ViViTest software handles experimental determination of the static head pressures as part of the pressure calibration routine.
- Alternatively, these static head pressures may be manually input into the software. When the test fluid is saline, the approximate values to be input are -6.5 mmHg for the aortic pressure site and -0.2 mmHg for the straight left atrium or -3.5 for the angled/rectilinear left atrium site. These values are referenced to the ventricle pressure measurement site, thus the ventricular pressure offset will be 0.0.

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8. System Preparation for Calibration

- Verify power is “On” for all instruments.
- Verify hardware and fluids:
  - Pump head (and VIA optional component) are fluid filled and debubbled;
  - Flow meter probe is in place and connected;
  - Atrium, Ventricle (silicone ventricular membrane is in a static state), Aortic Root and Aorta Chambers are fluid filled according to their specifications and debubbled;
  - Compliance Chambers are compliant;
  - Transducers are in place and connected;
  - Peripheral Resistance is connected;
  - Heat exchanger is connected (optional component).
- Verify electrical components and connections:
  - Pump controller is turned on and desired waveform control is selected. Ensure Limit is not illuminated (if it is, switch is moved in the direction of the arrow).
  - Flow meter turned on, MEAN is set to “HI”, ground cable is connected to Flow Probe pin. If the Alarm is illuminated depress and allow 10 minutes to stabilize. It is recommended to set the Range to 100 and Hz Response to 30Hz.
  - Pressure Amplifier turned on. Low pass filter settings set to recommended 30Hz.
  - Data Acquisition system turned on and ViViTest software loaded and running.

8.1 Pump Calibration – Operational Check

- Verify the **Pump Controller** is properly connected to all cable connections.
- The power supply is auto-detectable for 230/50Hz or 110/60Hz.
- Switch power button located on the back panel to “ON”. Piston should immediately home to the central cylinder position.
- The LCD screen will display any errors and provide status of the controller.
- Using the waveform button located on the front panel, select the desired waveform. If using ViViTest, the “external” channel should be selected.
  Note: For further pump control features - please see the SuperPump AR series user manual.

NOTE: Avoid large piston strokes that could activate the pump limit switches. Note that if the push or pull limits of the pump are reached, the pump will automatically reset to the home position.

1 Pump Piston Transducer Calibration

The pump piston linear displacement is measured for each unit. The factor in mv/mm is provided by ViVitro manufacturing. Follow the ViViTest pump calibration step to ensure the correct Pump Calibration Factor is entered.

8.2 Drive Waveform Generator Operational Check

The Pulse Duplicator is operated by unique complex analog waveforms supplied by the analog output of the I/O Module. See the ViViTest User Manual for instructions.

NOTE: Do not change the waveform setting while the pump is in operation. Set amplitude to zero before changing the waveform.

The selected waveform will dictate the operation of the pump to a prescribed push/pull (systole/diastole) ratio corresponding to a systolic duration percentage, characteristic waveform, and beat rate in beats per minute.

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8.3 Calibration of Flow Probe Methods

There are two distinctive methods to collecting flow measurements. Each should be considered prior to starting, so that the proper set-up and recognition by the ViViTest software is performed.

1. **Direct Flow** – This is the most common type of measurement. The flow probe should be mounted in the position of interest, i.e. aortic valve flow measurement requires the flow probe to be mounted on the Aortic Chamber side of the Heart Model. The ViViTest software will automatically determine flow for the probe site based on the target probe site and target valve. If these entries are the same the software will recognize the system as configured for direct flow measurements.
2. **Derived Flow** – This type of measurement is used when the experimentalist notes a problem that is noticeable as leakage flow computing to a positive value. This can result when using the electromagnetic flow measuring system and a disturbance of the flow field around the flow probe occurs. The problem can be particularly noticeable when using mechanical valves where small leakage paths can give rise to relatively high velocity regurgitant fluid jets and consequent highly disturbed flow in the model flow channels.

   (Positive leakage can also be caused by excessive valve compliance during back pressure and modifications to the valve mounting should be considered as the first source to resolve the problem; derived flow is best used when no other solution is available to the investigator).

   The derived flow method calculates the flow for the target valve by placing the flow probe and supplied competent valve (i.e. no leakage) in the companion site and computing the flow from the dL/dt pump signal. Given the check valve is designed not to leak the ventricular flow or pump displacement can be assigned as the target valve flow.

   NOTE: The use of derived flow has been validated by comparing “derived” flow leakage rates with those obtained from leakage column measurements. This validation was made using a ViVitro Labs Pulse Duplicator System with left atrium and mitral flow channels in line. The method is only valid with **NO** compliance. The VIA and any air in the hydraulic chamber must be removed. The competent valve must be used in the companion site and the rectilinear atrium chamber. This method is not recommended for the mitral position or with vertical atrium chambers.

NOTE: The ViViTest software alternately uses the same prompts for Direct or Derived flow measurements. The ViViTest will provide flow measurements for both the aortic or mitral position regardless of the configuration, so your choice of target probe site and target valve determines if the measurement for the target valve is direct or derived.

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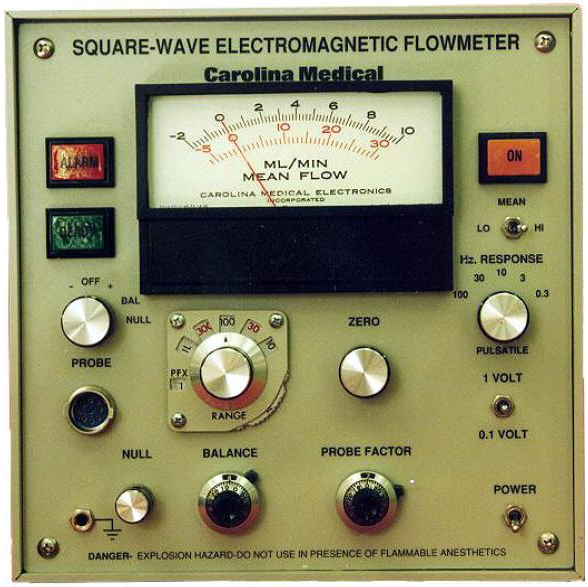
8.4 Flow Meter Operational Check

NOTE: Warning: Incorrect mains voltage setting may cause damage to the flow meter

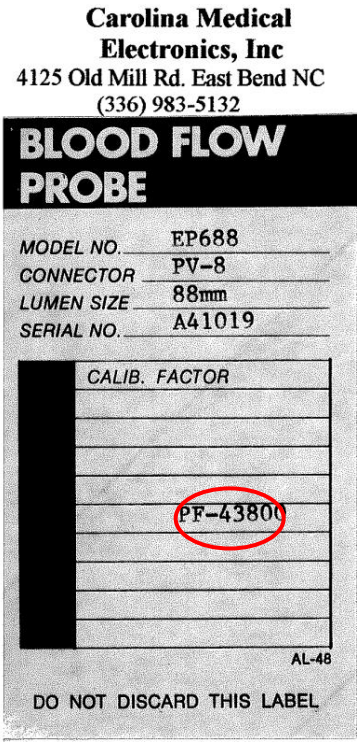


Installation Instructions:

- Refer to the Flow Meter Manual before changing the power plug style.
- Check that the voltage switch on the back panel of the unit is set for your mains supply (115 or 230 VAC).
- With POWER OFF (down) and PROBE switch OFF plug the power cord into the mains supply.

NOTE: The Flow Meter must be properly grounded.

- Connect PULS output on the back panel to the flow-monitoring device. A PHONO to BNC cable for connection to the I/O Module is supplied.
- Connect the 8-pin probe plug to the Flow Meter jack with the correct registration as indicated by the missing pin. The flow probe requires a conductive fluid such as saline to properly function. It will not function in air or pure water.
- Connect the ground jack on the front panel of the Flow Meter to the test fluid ground point corresponding to the flow probe site.
- Set the MEAN switch to HI.
- Set the outer dial on the RANGE switch to give a PFX (probe factor multiplier) of 1.
- The frequency response recommendation is 30Hz.
- The PROBE FACTOR should be set to the manufacturer's recommendation. See label below. The probe factor, shown in the red circle, translates to a setting of 4.38 on the flow meter potentiometer. Reference the probe factor calibration label provided with the flow probe for proper set-up.
  Following the ViViTest software instructions for flow calibration, the software will automatically adjust create a calibration factor for the flow meter signal to equal the pump dL/dt. If a significant drift is noticed in the flow meter conversion factor, the flow meter and probe may require calibration.



- Turn the POWER switch on. A switch transient may cause the ALARM indicator to illuminate. Depress the ALARM indicator to reset the meter. Allow ten minutes for the instrument to stabilize. The green READY light should remain on when the meter is measuring flow.
- With the PROBE switch set to OFF: Adjust the ZERO control to give a display meter a reading of zero.

  NOTE: The ZERO control is functional with or without the flow probe attached and the "no-signal" level of the back panel MEAN and PULS outputs are set by the ZERO control. The "with-signal" level should be approximately the same as the "no-signal" level under static flow condition.

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8.5 Null, Balance and Zero

NOTE: The following MUST be performed with the system filled with 0.9% NaCl (saline solution).

- With the PROBE switch set to OFF, adjust the ZERO control until the display meter reads zero.
- Set the PROBE switch set to NULL. Adjust the NULL control until the display meter indicates a MINIMUM reading.
- Set the PROBE switch set to BALANCE. Rotate the BALANCE control so that the meter reads zero. This position should be close to that set by ViVitro Labs Inc during pre-shipment calibration.
- Set the PROBE switch set to either + or – (Normal placement of the probe, dictates flow is in the "+" direction) If necessary, use the BALANCE control until the meter shows zero.

NOTE: If the meter is off scale, check for improper grounding or air bubbles in the probe lumen which can be removed using a cotton swab.

- To verify the flow meter is set and/or operating correctly, use the ViViTest software flow calibration screen to verify an oscillatory flow when the pump is operating. Reference the flow meter manual if the flow meter is suspected of not functioning properly.
- Turn the PROBE switch OFF when flow is not being monitored or NO test fluid is present.

NOTE: Warning: Probe can be damaged if left on when NO test fluid is present.

- Calibration of the Flow Meter may now be performed using the ViViTest software.

8.6 System Check

The ground jack on the front panel of the Flow Meter should be connected to the test fluid ground point corresponding to the flow probe site (see cabling schematic). If the fluid ground point is changed, the Flow Meter should be re-adjusted per steps starting at the NULL step.
When the Flow Meter is supplied by ViVitro Labs Inc, calibration of the unit using the ViViTest software is carried out prior to shipment. The calibration applies ONLY when the front panel control settings are those on the calibration sheet. The calibration sheet gives the calibration factor in units of mV/(ml/s). This may be used for manual input to the ViViTest software, however it is recommended that the flow meter be calibrated daily. The calibration sheet compares the volumetric flow rate generated by a piston pump and the resulting flow meter signal.
During calibration of the flow transducer no air must be in the VIA. The ViViTest software will equalize the flow meter output to the instantaneous net ventricle volumetric flow rate generated by SuperPump using a sinusoidal waveform at 70 or 100bpm. This volumetric flow rate is obtained by using the piston displacement signal differentiated with respect to time. This signal is output from the SuperPump controller back panel through the output labeled dL/dt. The dynamic flow transducer calibration is performed with no valves in the Model Heart. To take the place of disc valves, the accessories kit contains a silicon rubber flow straightener sealing ring and also a solid seal. The solid seal assures zero flow in the occluded site.

NOTE: After filling/refilling with test fluid, the meter should be re-adjusted as outlined in steps starting from NULL (4.3.2)

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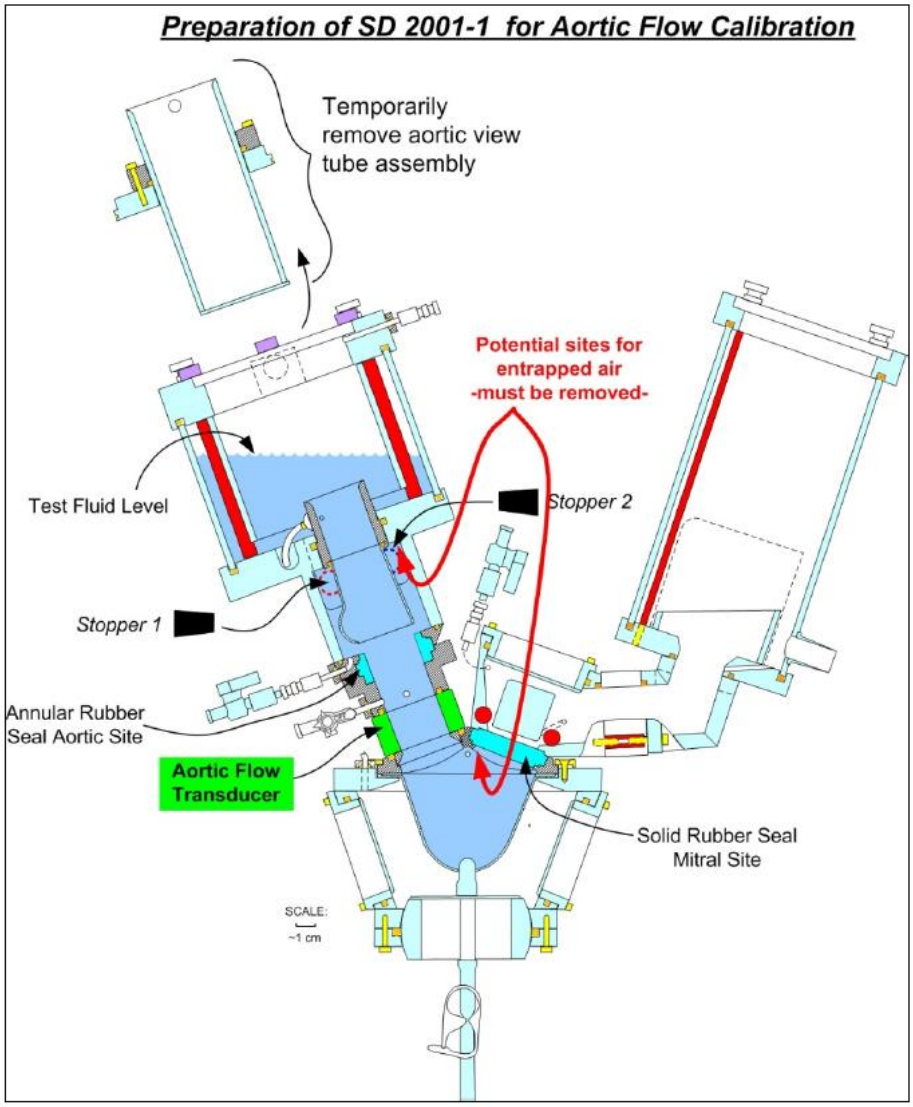
8.7 Calibration Procedure for Aortic Flow Probe

The diagram below illustrates the proper set-up for Aortic Flow calibration. Stoppers may be used as shown in the diagram or the tubing can be clamped. If the stoppers are used care should be taken to remove the tubing without damaging or breaking the acrylic hose barbs.


Preparation of SD 2001-1 for Aortic Flow Calibration
Temporarily remove aortic view tube assembly
Potential sites for entrapped air -must be removed-
Test Fluid Level
Stopper 2
Stopper 1
Annular Rubber Seal Aortic Site
Aortic Flow Transducer
Solid Rubber Seal Mitral Site
SCALE: ~1 cm

It is recommended that the flow probe be calibrated using pulsatile flow generated in the model. This can readily be done using the SuperPump with no air in the hydraulic chamber and VIA. The instantaneous net ventricle volumetric flow rate should equal the flow rate generated by the SuperPump. The ViViTest software will facilitate the sequence of steps to determine these measurements and determine the calibration factor. A sine wave from 70 -100bpm is required to create a stable oscillating flow. The previously calibrated pump volume dL/dt signal is matched to the flow probe signal.

To proceed with aortic flow probe calibration:

1. Insert probe in aortic site as described previously;
2. Remove window assemblage on top of aortic chamber (loosen bolts holding three retaining tabs, rotate tabs to window release position, and pull up on window;

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3. Occlude RED and BLUE ports on aorta housing block with rubber stoppers or clamp the tubes securely and clamp drain hose;
4. Place silicon rubber annular seal in aortic site and secure the aortic chamber with thumbscrews;
5. Occlude the mitral site with solid silicon rubber seal;
6. Place atrium assembly over solid seal and secure by tightening the four thumbscrews;
7. Add fluid (must contain 0.9% NaCl) to load reservoir until level just reaches mitral site. Continue to fill the system through the open top on aortic chamber reservoir until the test fluid level reaches the level indicated in the diagram on above;
8. Remove all entrapped air through ports occluded by stopper 1 & 2 (may be necessary to tilt model).
9. It is important to remove all air from the aortic root assembly.
   This can be done via the BLUE port. It may be necessary to tilt the model so that all air can exit through this port. Switch on the drive to SuperPump and record the piston displacement, piston displacement differentiated with respect to time (dL/dt), and flow transducer signals. Using the ViViTest software following the FLOW Calibration steps. The phasing between these signals can also be determined. If the electronic filters on the instruments are matched (typically, either 30 Hz or 100 Hz low pass filters) then any measurable phase difference for these signals is likely due to the characteristics of the transmission path between the flow generator (piston) and the flow probe.

NOTE: Always verify the flow zero, reset electronically by following the software prompts for re-zero.

10. Turn the pump controller amplitude to the desired pump stroke volume; typically 70ml.
11. Verify the proper flow meter PROBE FACTOR value obtained from the manufacturer calibration label is set;
12. The system is now ready for flow calibration in ViViTest.

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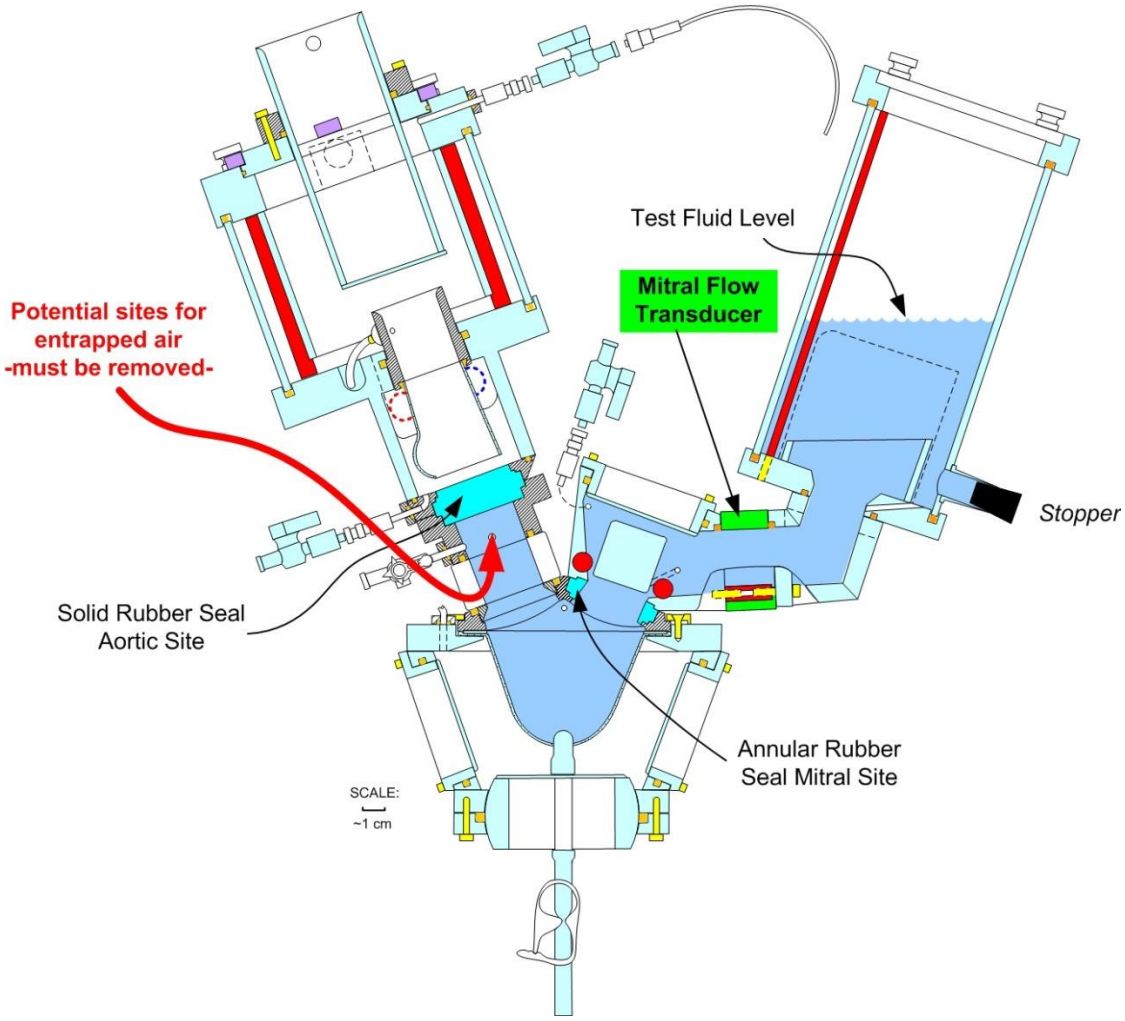
8.8 Calibration Preparation of Mitral Flow Probe

The diagram below illustrates the proper set-up for Mitral Flow calibration. Stoppers may be used as shown in the diagram or the tubing can be clamped. If the stoppers are used care should be taken to remove the tubing without damaging or breaking the acrylic hose barbs.



Mitral flow calibration is conducted with the same principals as those previously described for aortic flow calibration. Similarly, set-up the model with no air in the pump head and VIA. The instantaneous net ventricle volumetric flow rate should equal the flow rate generated by SuperPump. This flow rate is obtained by using the piston displacement signal differentiated with respect to time.
This is output from the SuperPump power amplifier back panel through the output labeled dL/dt. The dynamic flow probe calibration is performed with no valves in the system. To take the place disc valves, mounting rings from the accessories kit contains a silicon rubber flow straightener sealing ring and also a solid seal for the aortic valve non-test site.

To proceed with mitral flow probe calibration preparation:

1. Insert flow probe in mitral site as described in the installation section;
2. Place silicon rubber annular seal in mitral site;
3. Place atrium assembly over seal and secure by tightening the four thumbscrews;

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4. Use black rubber stopper to occlude the green hose port or clamp securely and clamp drain hose;
5. Add fluid (must be 0.9% NaCl) to the atrium reservoir until fluid reaches aortic valve site;
6. Occlude aortic site with solid silicon rubber seal without trapping air beneath seal;
7. Place load assembly over solid silicon rubber seal and secure by tightening thumbscrews;
8. Complete filling of chamber until the test fluid level reaches the level indicated in the diagram above;
9. Switch on the drive to SuperPump and record the piston displacement, piston displacement differentiated with respect to time (dL/dt), and flow probe signals. Using the ViViTest FLOW Calibration steps, the calibration of these signals can be conveniently determined. The phasing between these signals can also be determined. If the electronic filters on the instruments are matched (typically, either 30 Hz or 100 Hz low pass filters) then any measurable phase difference for these signals is likely due to the characteristics of the transmission path between the flow generator (piston) and the flow probe.
10. Turn the pump controller amplitude to the desired pump stroke volume; typically 70ml;

NOTE: Always verify the flow zero, reset electronically by following the software prompts for re-zero.

11. Verify the proper flow meter PROBE FACTOR value obtained from the manufacturer calibration label is set;
12. The system is now ready for flow calibration in ViViTest.

A CAUTION about FLOW Measurement
Electromagnetic flow probes (e.m.probes) are best suited to measurement of uniform streamline flow (non-turbulent). Flow in Model Heart channels may deviate considerably from ideal for e.m. probes, particularly during the leakage flow phase when regurgitant jets from valves produce unfavorable flows in the probe lumen. This can be most problematic at high cycle rates and flows. The flow signal must therefore be interpreted advisedly. In particular, a positive flow signal seen during the leakage phase for a valve known to seal is indicative of an erroneous flow signal. This problem may be reduced by rotating probe (or valve) about the central flow streamline to minimize direct impingement of jets on the electrodes. Additionally, a rigid or less compliance mount can reduce the effects of positive leakage.
Another solution for this flow measurement problem is to use indirect flow measurement. For a leaking valve in the aortic test site, the alternative method of measuring flow is by placing the flow probe in the mitral site and eliminating any compliance between pump and ventricle. In this way, the pump displacement differentiated with respect to time (dL/dt) becomes the ventricle net volumetric flow rate. In this case, with the e.m. probe in the mitral site, impingement of jets on electrodes should be less problematic because of the mitral flow channel configuration.
The ViViTest software handles synthesis of the derived aortic flow signal from the e.m. probe mitral site flow signal and the pump dL/dt.

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8.9 Calibration of Pressure Measurement System (AP9991) Initial Setup

1. Switch the unit ON (rear panel). Allow the amplifiers to warm up for 30 minutes to achieve minimum drift.
   NOTE: Warm up time can be avoided by leaving the unit on continuously. This will not damage the system.

Select the desired filters using the filter switch located on the front panel of each amplifier. For pulse duplicator measurements 30 Hz is recommended.

- INPUT: always use the locking screws on the cable connector.
- GAIN: adjustable by 15 turn potentiometer.
- FILTERS: A five position switch selects upper frequency cut-off. Normally this should be lower than the natural frequency of the transducer.
- SHIFT: 15-turn potentiometer; each turn shifts output 2.5 volts between full scale ±12 volts.

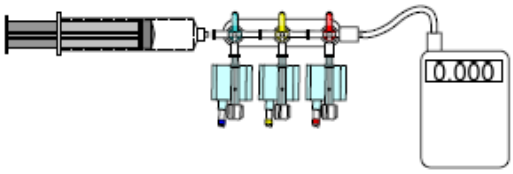
For additional specifications or instructions refer to the Pressure Measurement User Manual.

GAIN & SHIFT Adjustment and Calibration

Each amplifier module and pressure transducer may be calibrated following the procedure given below.

1. Using a Manometer, an appropriate range and accuracy, and a volt meter with a display of -10.000 and +10.000 volts DC to an accuracy greater than the pressure gauge accuracy.

Follow the ViViTest software calibration steps, inputting range (i.e. 0-200mmHg), recognition of atmospheric pressure voltage (-6.0 volts; adjust SHIFT as needed), and recognition full scale voltage (6.0 volts; adjust GAIN as needed).



NOTE: To avoid poor signal to noise ratio and recording of erroneous data, the full scale capability of recording instruments should be matched to the range of the dynamic signals to be measured.

Use a trim-pot tool provided in the Accessories Kit to adjust the 15-turn potentiometers for GAIN & SHIFT. The extreme position, indicated by a click, may be located by rotation of at least 15 turns clockwise or anti-clockwise. A two point pressure transducer calibration method can be made as follows:

- Connect amplifiers to pressure transducers and switch amplifier ON.
- Use a syringe to fill the pressure transducers with distilled water.
- Adjust the SHIFT control to give -6.0 volts output at 0 mmHg gage pressure (atmospheric).
- Apply a pressure of +200 mmHg to transducers and adjust GAIN for +6.0 volts output.
- Repeat steps 4 and 5 to achieve the stated outputs.
- CALIBRATION = voltage/pressure = 12 V/200 mmHg = 0.06 V/mmHg.

NOTE: The GAIN and SHIFT settings may need to be changed depending on the range of pressure generated in the specific application. Different settings may be needed for monitoring the ViVitro Labs Inc. Pulse Duplicator System. Changing GAIN will also change the output at atmospheric pressure. It may be necessary to re-adjust SHIFT until the required outputs are obtained at the two calibration pressures.

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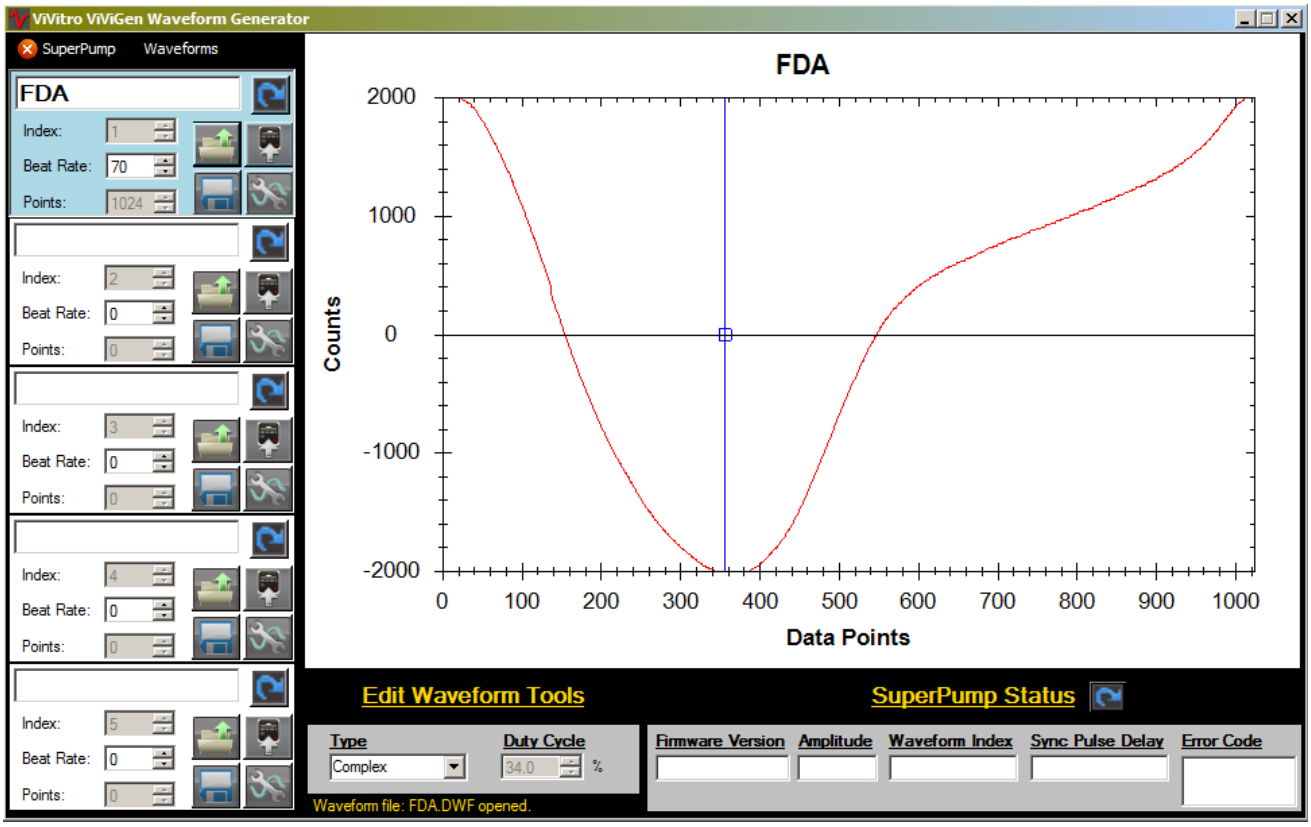
9. ViViGen Software



9.1 Installation

Install the ViVigen Installer.exe on the computer. This executable program can be found on the supplied flash drive. During installation setup, check the box “Create a desktop icon.”

9.2 Method

Connect the SuperPump Amplifier to the computer using the USB cable.

Turn on the amplifier.

Open the ViViGen shortcut icon on the desktop.

Click the

label on the upper left corner to access the drop-down menu.

Select “Connect...” and select a “COMX” value.

If the connection is successful, the red dot will turn green.

Click the

label on the upper left corner to access the drop-down menu.

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Select “Refresh Waveforms” to upload current waveforms from the amplifier to the Waveform Generator console.

The amplifier and the console are able to access five waveforms.

9.3 Icons

The left side of the console panel shows five index positions. These correspond to the five waveforms stored in the amplifier. It is possible to modify these waveforms using the four icons and the drop-down boxes for each position.

Use the “Open File” icon to search for a stored waveform in the software package. Open the file and view the waveform on the graph.

Use the “Tools” icon to modify or edit the waveform, its label or its Beat Rate. Be sure to define the Type and Duty Cycle, under the “Edit Waveform Tools.” Note: The “Beat Rate” has a max value of 400. Do not use a beat rate higher than 220 when running the SuperPump.

Use the “Save” icon to save the modified waveform to a file.

Use the “Refresh” icon to view your changes. Note: Remember to save your waveform, before using the “Refresh” icon, when making changes under “Edit Waveform Tools.”

Use the “Send” icon to send the waveform to the amplifier.

Note: Do not attempt to “Refresh” or “Upload” a waveform when the SuperPump is running.

9.4 Status Request

Use the “Refresh” icon beside the “SuperPump Status” label to update the screen view with the current status of the pump. Note: the amplitude number represents a percentage value, not a stroke volume.

Waveform index gives the index number of the waveform currently being used by the amplifier. Sync pulse delay is given in milliseconds, and can be adjusted using the amplifier controls.

Observe the error code box, under the “SuperPump Status.” Any communications failures or other problems will be signalled here. Note that an attempt to communicate with the amplifier will be repeated until successful or the software is terminated.

Note: If the USB is disconnected and then reconnected, the “Refresh Status” will not update. The SuperPump must be reconnected using drop-down menu, in upper left corner.

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9.5 New Waveform Generation

New waveforms may be created using the drop-down menu from the Waveforms label in the upper left corner.

Select “New” and complete the requested data in the “Create New Waveforms” window. Note that 1024 is the standard and expected number of Points.

Enter its Label name, its Beat Rate, and its Waveform Type.

Select the “Save” icon and name the file appropriately. Select “Done” when completed.

The new waveform may now be accessed from one of the five index positions, and the duty cycle and beat rate adjusted as required.

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10. ViViTest Software Operation

1. Set up the Pulse Duplicator System (refer to previous sections).
2. Double click on the ViViTest icon located on your desktop.
3. Select the preferred calibration file. (Calibration.cfg is the ViVitro Labs' default file with default values to run the software.)

NOTE: Good practice indicates that to verify the configuration of the system users should test a valve with known characteristics at the start of every session.
Once users are familiar with the waveform image associated with a standard valve, inconsistent waveforms from test valves can be quickly recognized even before analyzing numeric results.
The .ACQ data file associated with the standard valve can be loaded in either the Analysis or Review pages in the ViViTest application.
In most standard test situations waveform pressure ranges should correspond to physiologic pressure ranges.

10.1 Calibration Process Overview

NOTE: It is advised that calibration is carried out each time the software is restarted and at key intervals during use.

NOTE: First time through, perform Pump calibration FIRST.

Users can select any of 4 types of calibration to perform:

Measure sensor zero references

Calibrate the flow meter and flow probe(s) (Mitral/Aortic)

Calibrate and zero the 3 transducer pressure sensors (atrial, ventricular and aortic)

Measure pump linear displacement

A complete calibration involves going through each of these Calibration Wizards which is outlined in the following sections.

NOTE: The calibration values are read from the selected calibration file and are represented within the Session Information Panel and the Calibration Wizard. These values can be altered to reflect the desired values and saved to a new calibration file via the Calibration Wizard.

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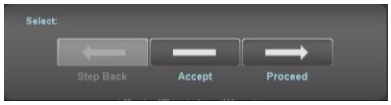
10.2 Calibration Wizard Navigation

The Calibration Process is a step-by-step process guided by the Calibration Wizard. The wizard explains each step of the process leading to measured offsets or gains that constitute the calibration values shown in the Sensor Calibrations panel on the right hand side of the screen.
Each of the calibration processes has a navigation panel where users have the opportunity to proceed to the next step or accept the values as presented. Should previous steps need to be revised the user may repeat the process or alternately take a step back to revisit the data entered.



Close — At any time during the calibration process users can abort by selecting the Close button. This immediately cancels the calibration sequence without saving any of the newly input values.

Collapse — The Collapse button enables the user to momentarily stall the calibration process without losing the values entered. It causes the Wizard to reduce to a thumbnail in the left hand corner of the screen.

Wizard Thumbnail — Once the Collapse button is selected a thumbnail will appear in the bottom left corner of the screen. Selecting this thumbnail returns the Wizard to its full size once again.

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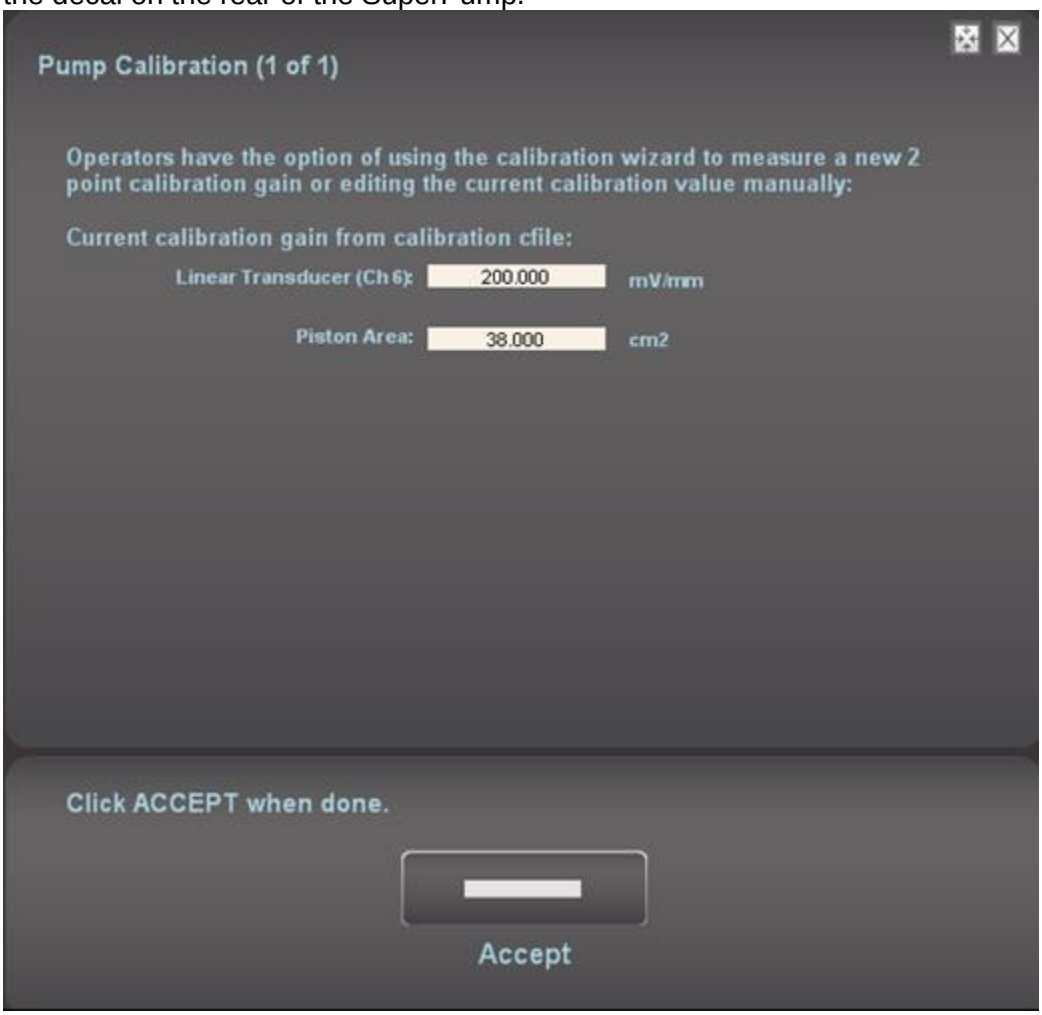
10.3 Pump Calibration

The pump piston linear displacement is measured for each unit. The factor in mv/mm is provided by ViVitro manufacturing. The value as shown below is used by the ViViTest software to calibrate the pump. The Linear Transducer (Ch 6) value should be pre-set from ViVitro between 195 and 199, follow the ViViTest pump calibration procedure in the Appendix to ensure the correct pump calibration Factor is entered. The Piston Area should match the area listed on the decal on the rear of the SuperPump.


Pump Calibration (1 of 1)
Operators have the option of using the calibration wizard to measure a new 2 point calibration gain or editing the current calibration value manually:
Current calibration gain from calibration cfile:
Linear Transducer (Ch 6):
200.000
mV/mm
Piston Area:
38.000
cm2
Click ACCEPT when done.
Accept

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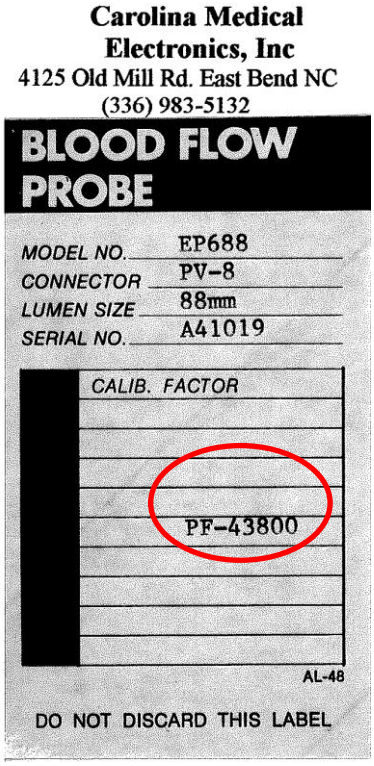
10.4 Flow Meter – Calibrating the Flow Meter

With this calibration, the flow rate through the transducer will be made directly proportional to the piston displacement signal differentiated with respect to time (dL/dt). The latter signal is an output from the pump amplifier.
The PROBE FACTOR should be set to the manufacturer's recommendation. See Carolina Medical label. The probe factor, shown in the red circle, translates to a setting of 4.38 on the flow meter potentiometer. Reference the probe factor calibration label provided with the flow probe for proper set-up.



Following the calibration wizard instructions, the software will automatically adjust to create a calibration factor for the flow meter signal to equal the pump dL/dt. If a significant drift is noticed in the flow meter conversion factor, the flow meter and probe may require calibration.
Three zero crossovers for each signal should be seen on the voltage graph on step three of the Wizard within the Process Display area. Where this is not the case an error window will appear to run the user through the process again.
A typical Pulse Duplicator System configuration consists of a single flow sensor so dL/dt is the default selection meaning that overall system flow is calculated using the pump's RPM sensor, and that flow at the 'un-instrumented' side of the model will be indirectly derived from both Channels 4 and 5.

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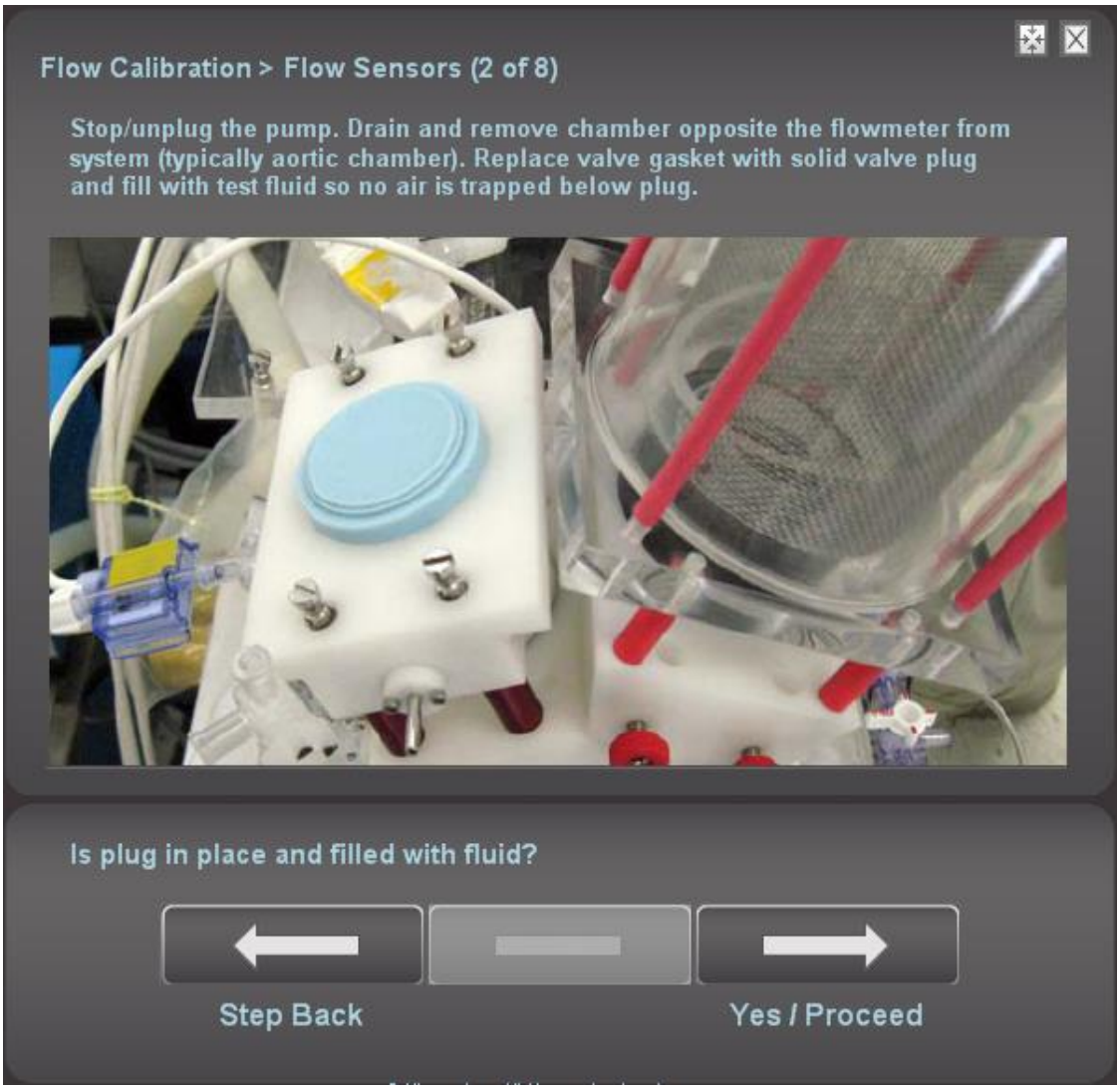
Flow Calibration > Flow Sensors (2 of 8)
Stop/unplug the pump. Drain and remove chamber opposite the flowmeter from system (typically aortic chamber). Replace valve gasket with solid valve plug and fill with test fluid so no air is trapped below plug.
Is plug in place and filled with fluid?
Step Back
Yes / Proceed

1. Set “Pump Control” settings on the right to desired levels (Typically users would use a cycle rate at 70bpm using a sine waveform of 50%). A sine wave is required for accurate flow calibration.
2. Click “Flow” under calibration stage on left
3. Follow step by step calibration wizard

NOTE: The data summary presented at the end of the calibration collection sequence is used to show the adjustment (k-factor) of the flow channels to the dL/dt channel. It is important to determine internal acceptance criteria for the flow channel calibration. The software automatically adjusts the flow channel k-factor to equal the dL/dt channel.

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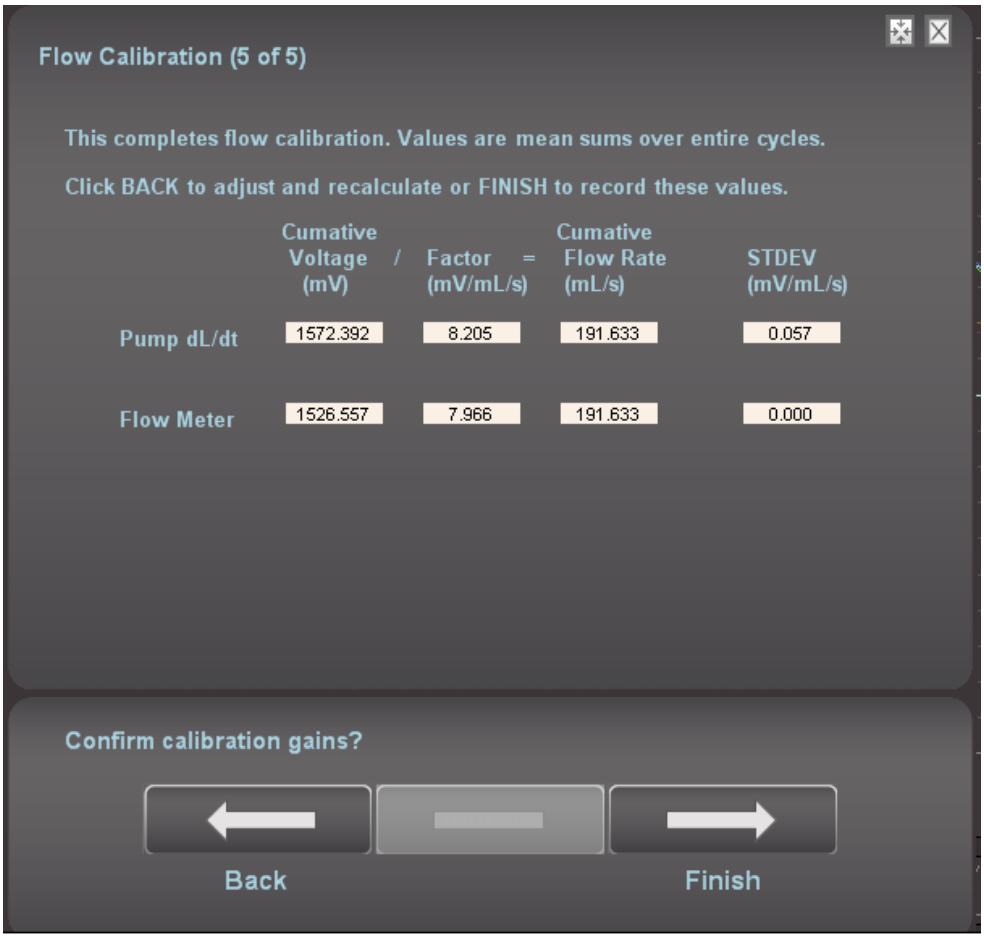
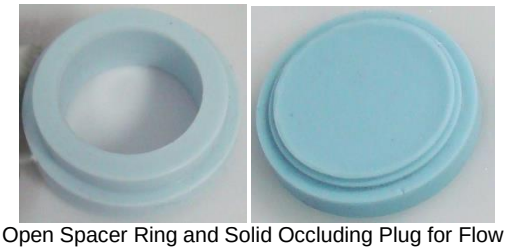


NOTE: To calibrate the flow transducer using the ViVitro Labs Pulse Duplicator System place an open spacer ring in the flow probe site and an occluding solid plug located in the opposite site. These items are provided in the Pulse Duplicator Accessories Kit. (There should be no compliance between pump piston and flow probe).



ViViTest – Open Spacer Ring and Solid Occluding Plug for Flow Calibration

In cases where flow transducers are mounted in both the aortic and mitral position, the dL/dt signal can be ignored in favor of direct flow measurement at the valve.

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10.5 Pressure Calibration – Calibrating and Zeroing the Sensors

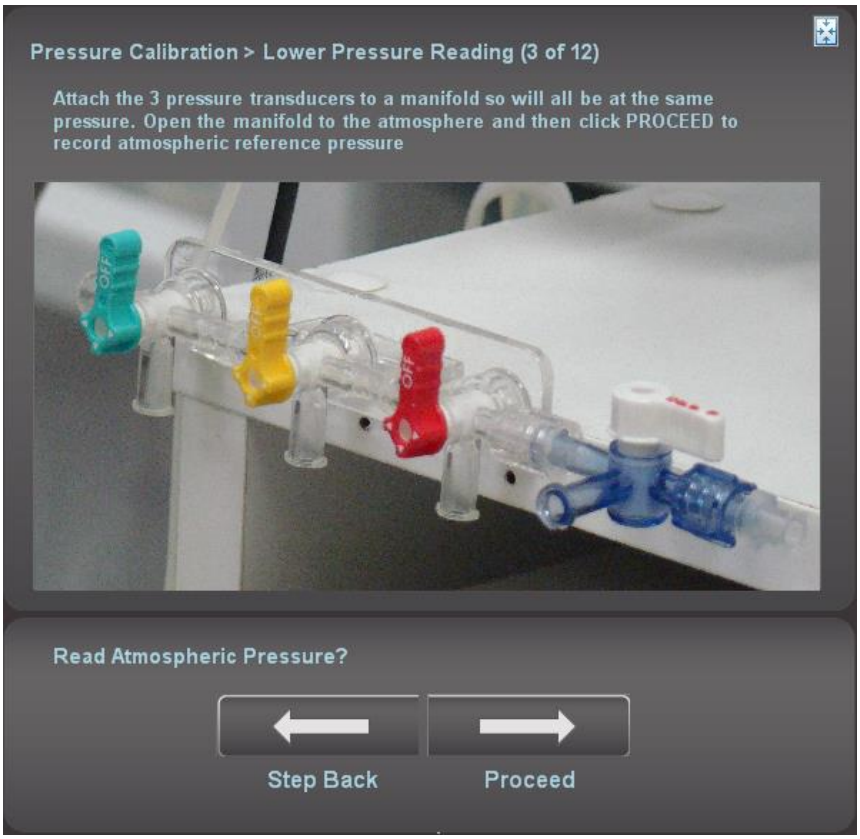
Calibration of the aortic, ventricle and atrial pressure sensors has two parts:

- a 2 point pressure calibration, then
- a measurement of static pressure head differences between the 3 pressure transducers.

NOTE: 2 point pressure calibration pressures must be applied to all 3 transducers simultaneously.

NOTE: The low and high pressures should be chosen to span the expected working range of the transducers, e.g. 0 to 200 mmHg.

NOTE: Usually the 3 transducers are mounted on a manifold and first exposed to the atmosphere to establish the lower end of the range. Then a large syringe and a digital manometer are used to apply the upper range pressure. The software measures the ADC voltage at each of these limits and uses these as a 2 point slope in calculating the calibration gain.


Pressure Calibration > Lower Pressure Reading (3 of 12)
Attach the 3 pressure transducers to a manifold so will all be at the same pressure. Open the manifold to the atmosphere and then click PROCEED to record atmospheric reference pressure
OFF
OFF
Read Atmospheric Pressure?
Step Back
Proceed

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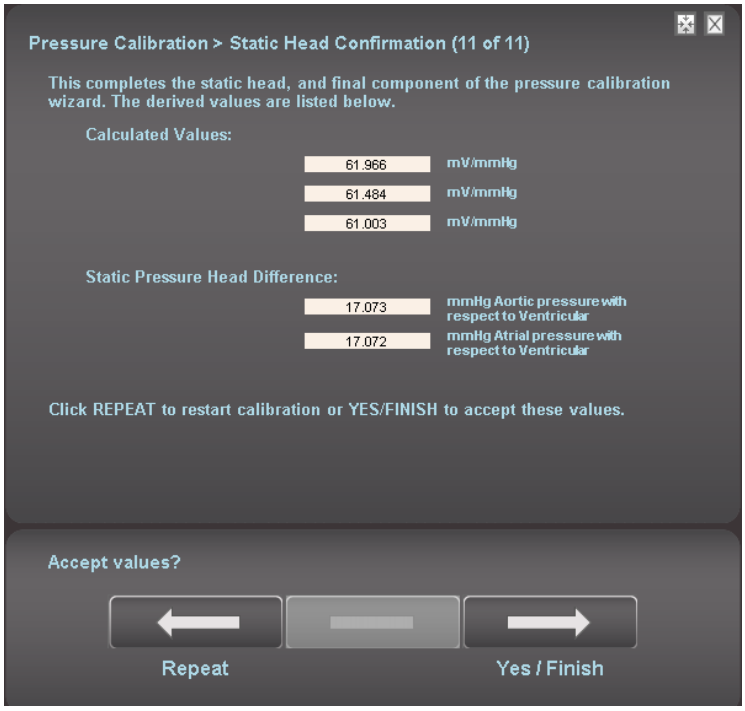
Pressure Calibration > Static Head Confirmation (11 of 11)
This completes the static head, and final component of the pressure calibration wizard. The derived values are listed below.
Calculated Values:
61.966
mV/mmHg
61.484
mV/mmHg
61.003
mV/mmHg
Static Pressure Head Difference:
17.073
mmHg Aortic pressure with respect to Ventricular
17.072
mmHg Atrial pressure with respect to Ventricular
Click REPEAT to restart calibration or YES/FINISH to accept these values.
Accept values?
Repeat
Yes / Finish

In the ViVitro Labs Pulse Duplicator System, wall pressure measurement sites are not at the same level. Experimental determination of the static head pressure differences is the second part of the pressure calibration. The Wizard prompts the user to record pressure transducer outputs when they are once again installed in the model:

- first with atmospheric, reference pressure applied, and
- then with the static head of fluid pressure applied.

Application of these pressures is readily made by turning the T taps on the transducers. For custom systems, where pressure transducers are at the same head level, zero static head pressure offsets can be entered manually.

NOTE: The calibration factors presented at the end of the calibration collection sequence are related to the accuracy of the pressure data collection. It is important to determine internal acceptance criteria for the pressure transducer calibration factor. The integrity of your data and quality of the results are related to the degree of acceptable resolution. The data accepted should be reviewed to ensure the calibration specification meets the testing objectives.

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10.6 Zero Calibration – Measuring Sensor Zero References

Zero offset measurement is the most common of the calibration processes and best practice is that this be done frequently within a valve trial session.
Zero values for the sensors are calculated for the pressure, pump and flow sensors on a quiescent system open to atmosphere.
Users initially have the option of entering known values directly into the editable fieldsin the Calibration Wizard or follow instructions given by the Calibration Wizard to measure zero voltage values using ADC input.
The software tracks the changes of transducer zeros against previously stored zeros. These are indicated as the Drift value in the Sensor Calibrations panel.
NOTE: Abnormal zero DRIFT may indicate a malfunctioning transducer.

10.7 Finishing Calibration – Updating the Calibration File

After all the calibration processes have been completed select the Finished button to save the calibration parameters regenerated in the wizard. At this point you have the option to print the calibration report. This will be saved in the Files folder as an .htm file.
Whenever a new calibration is completed the file is rewritten and a new valid checksum calculated.

10.8 Acquisition Process

The Acquisition Process allows operators to capture 10 successive waveforms (cycles) for the valve being tested for subsequent analysis by the Analysis Process. The Process Display area displays pressures, flow, transvalve pressures, and pump stroke volume. The Process Controls modify the pressure display. The Support Information and Data Dashboard areas have a number of data boxes showing critical metrics such as cardiac output and pertinent pressures, volumes and flows.
NOTE: During the Live Acquisition Phase, voltages measured by the I/O Module are rendered as 6 pressure and flow traces in the Process Display area every ~2.5 seconds – each trace corresponds to an A-D channel. Users can hide unwanted traces by selecting one or more of the selector buttons along the left side.

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10.9 Standard Valve Verification

Adjust the Pulse Duplicator System until the quality of the waveforms shown in the graph panel is considered valid.
Complete the fields in the Session Information panel. It is particularly important that users indicate the location of the Flow sensor so that flow algorithms operate properly.

NOTE: Trial date is filled in automatically.

Click the

button to capture 10 consecutive valve pulse cycles
Save the captured data to an .ACQ file.

The Data Dashboard area shows the data for both the aortic and mitral parts of the heart cycle.
NOTE: Once users select the 'target valve' on the right, the opposite 'control' valve data is de-emphasized.

Snapshot

At any point users can select the Snapshot button in the Top Menu Bar to capture a .JPG image of the graph to record particular features of valve behaviour.

10.10 Re-zero Sensors

At any time during a test, users have the option of returning the system to a quiescent state and collecting sensor zeros. This is helpful in confirming sensor accuracy and drift.

10.11 Analyze Process

Once one or more 10-cycle ACQ files have been saved users can begin to perform a more comprehensive analysis of the valve cycle waveforms by selecting the Analyze tab – an open file dialog allows users to select a valve trial from the list of ACQ files contained in the C:\Program Files\ViVitro Labs\ViViTest\Files folder on the right.
The left side selector buttons allow users to select one, several or all of the 10 acquisition trial waveforms for graphing.

NOTE: When the All Waveforms (cycles) button is selected, statistics shown on the graph and in the lower results panels apply to the first waveform by default. To see the results of another waveform, simply click once on the waveform number you wish to see. When some but not all of the waveforms are selected statistics apply to the most recent waveform selected. In all cases data in the results panel is a mean of 10 cycles. The [data table] contains details for each waveform.

Data table button located at the top of the screen

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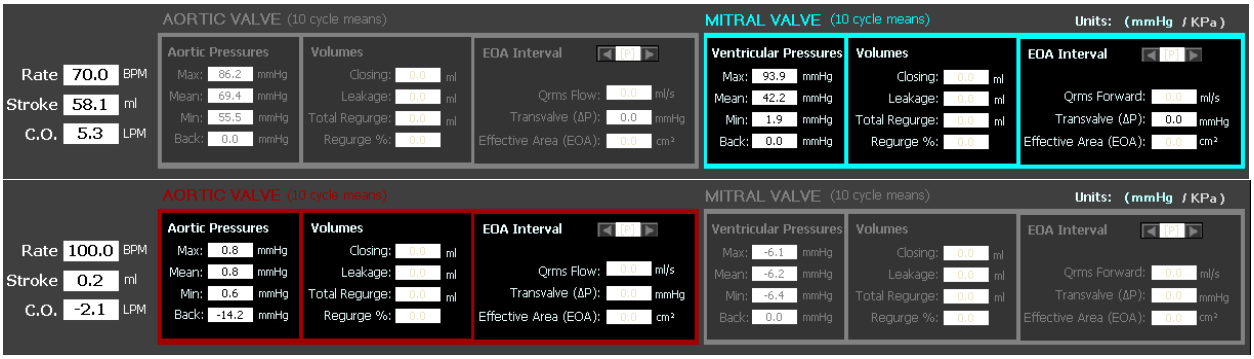
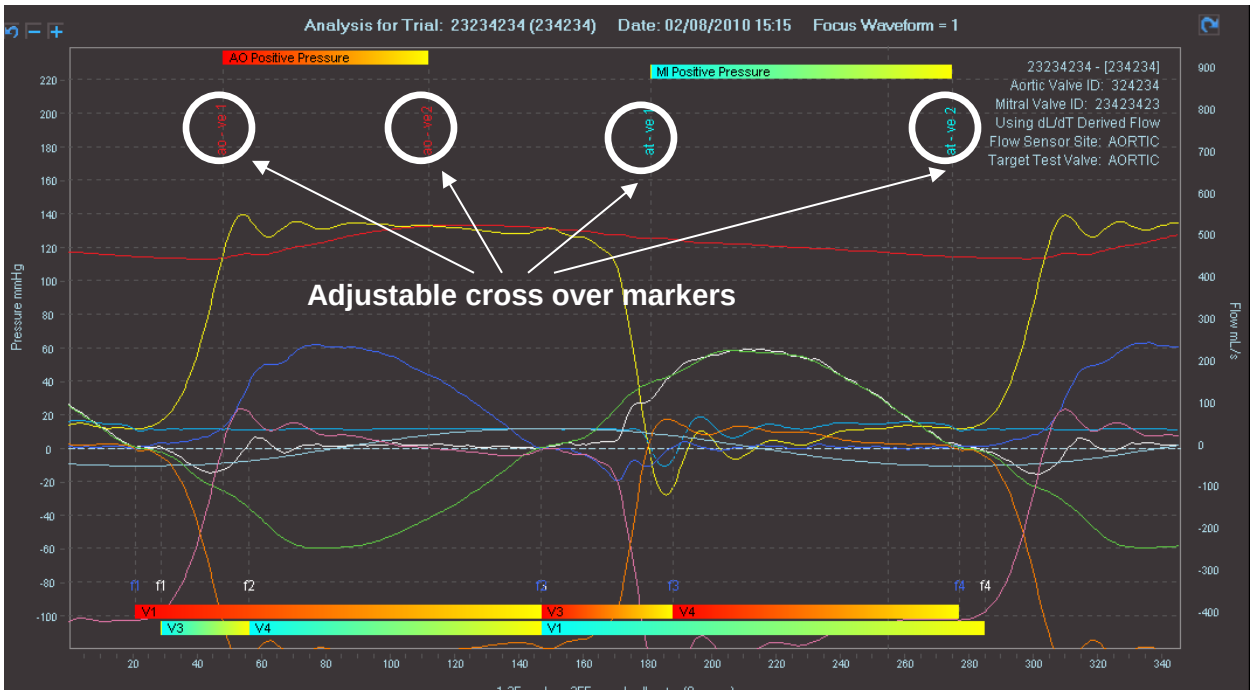
10.12 Crossovers and Interval Identification

Data analysis proceeds sequentially from cycle 1 to 10. Pressure and flow waveforms are displayed along with crossover markers defining critical intervals during the cycle. Automatic algorithms calculate the start and end of the aortic and mitral valve pressure and flow cycles. The Data Dashboard is based on these 'crossover' points.



NOTE: While the algorithms make reasonably good choices on well formed waveforms do not blindly rely on this automatic placement. Manually adjust the crossover markers by clicking and dragging on their labels to change their horizontal location to get best results.



Overlaid gradient 'bars' at the top of the graph mark pressure intervals - bars at the bottom of the graph mark flow intervals.

NOTE: Markers must be in correct logical sequence from left to right. If this is not the case, analysis will stop and an error bar will appear below the graph. Markers must then be moved to be in sequential order.

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The end of the Closing Flow interval may need adjustment, particularly if the flow is non-zero at these points. There may be a precursor to positive flow in which case the start of forward flow could be advanced in time to avoid negative values of mean transvalve pressure during the forward flow interval.
Users should check the crossover placements for each of the 10 waveforms that makeup the analysis data.
NOTE: When "All Waveforms" is selected, moving markers will affect all 10 waveforms.

10.13 Producing Data Output

Once analysis and adjustment of 10 cycles of data has been completed, means and standard deviations for the 10 cycles can be output as an .ANA file, .CSV file, data table or printed report by selecting the Save, Export CSV, Data Table or Print icons (see the Appendices for further instruction).
As in the Acquisition Process, graph traces may be hidden by selecting their trace button below the graph. Users can define the graph's level of complexity by selecting trace names in the Pressures and Flows Legend to highlight or hide traces.

10.14 Derived Flow Measurements

Total System Flow – Flow Meter Flow = Un-instrumented Valve Flow

This derived flow will only be meaningful if the data was acquired using the ViVitro Labs Pulse Duplicator System with no compliance between pump piston and the model's ventricle. In this case, the derived flow should correspond to the flow in the side without a flow probe, either aortic or mitral.

10.15 Useful Icons

Refresh Use the refresh button in the upper right corner of the graph to clear any artifacts from the graph should it become corrupted.

To change pressure units for the values shown in the Data Dashboard, select the mmHg / KPa toggle at the upper right corner.

Static head pressure compensation is one of the parameters measured during the sensor calibration process. Users can enable or disable these 3 pressure sensor compensation values to the data algorithms by selecting the Static Head compensation checkbox in the Sensor Calibrations panel.

Save File Once satisfied with the results, save the Analysis means for the 10 waveforms to an .ANA file by selecting the Save button.

Data Table Users can review the results calculated for each of the 10 waveforms in detail by reviewing a data table showing all 10 waveforms and the cumulative means. Select the Data Table button in the title bar to bring up the data table.

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Export CSV To put the same calculated results into an editable file for later off-line analysis select the Export CSV icon – this creates a comma separated value (CSV) file.

Printed Report Users can create a report of the Analysis by selecting the Print icon to write an HTML file to the Files folder. Verify the data shown in the on-screen report review.

NOTE: Files generated by the software such as CSV files or HTML report files are written to C:\Program Files\ViVitro Labs\ViViTest\Files unless otherwise directed.

10.16 Compare Process

The Compare Process allows users to compare analysis for a series of valve trials:

- for one valve
- against a similar series of trials for a second, third, … valve.

Features of the Compare Process interface:

- Main Process Control select categories of mean data to be plotted,
- Various Y axis data types can be selected by selecting the direction buttons flanking the Y axis label,
- Various X axis flow data types can be selected by selecting the direction buttons flanking the X axis label,
- The range of the X and Y axes are controlled by the zoom buttons at the right or top end of these axes,
- The Data Dashboard is split between a pair of trials selected from 2 groups.

The following data types may be shown:

Area - Graph displays curve of selected groups for Area (P - pressure), (PF – pressure and flow), (F - flow)

Energy - Graph displays curve of selected groups for Energy (Forward), (Closing), (Closed), (Total), in mJ.

Pressure - Graph displays curve of selected groups for Pressure (Mean Back), (Peak Differential), (Mean Positive), (Mean Differential forward), (Mean Differential hybrid).

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Regurge - Graph displays curve of selected groups for Regurgitation (Total), (Percent), (Closing), (Closed), and (Leakage Rate).

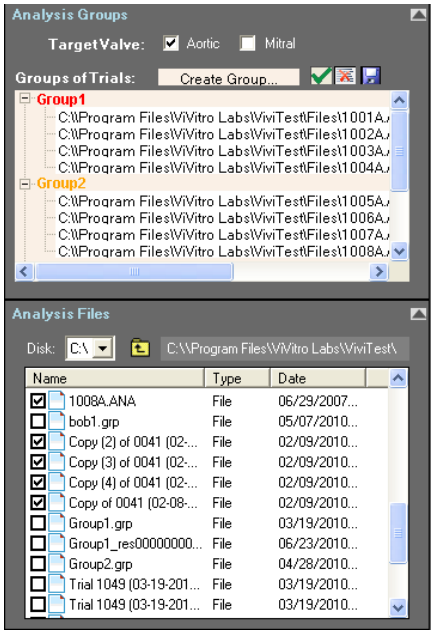
NOTE: There is no Legend bar for this interface. See the help screen for more information.

10.17 Comparing Groups of Trials

Groups are created by selecting the Groups of Trials buttons in the top of the Analysis Groups panel at screen right.
The sequence of creating groups for comparison is as follows:

- select either aortic or mitral Valve Data,
- select the checkbox to of each .ANA file required to create a group with a default name or edit the 'Create Group' box for a custom name ,
- add ANA files to the group by selecting the checkbox next to the file name in the Analysis Files list,
- repeat the steps above to create another group,

NOTE: If the groups tree need to be cleared select the Clear button .



Save the group using the save button and enter a file name in the Save dialog box. The new 'groups' file appears as a .GRP name in the Analysis files list.

10.18 Auto-scaling and Zooming

When users select a different Y graph type or change the data type selection shown in the X or Y axis the graph is auto-scaled to fit the data before it is rendered. Users can override and adjust this auto-scaling using the zoom buttons for either axis.

10.19 Handling Compare Results

Export CSV

To put the list of trial means being compared into an editable file for off-line analysis select the Export CSV button – this creates a comma separated value (CSV) file.

Print Report

Users can create a report of the Compare means by selecting the printer icon in the title bar to write an HTML file to the Files folder. Verify the data in the report review and either delete the HTML file or print to the system printer from a browser.

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10.20 Review Process

The Review Process allows users to browse acquisition (.ACQ) files and quickly graph their pressure, flow and differential transvalve pressure and power characteristics on a common timescale.
The list of current .ACQ files is shown in a checked list in the Selected Files panel at screen right.

10.21 I/O Module Re-initialization

In cases where the I/O Module needs re-initialization the following sequence is recommended:

1. Remove the I/O Module USB cable from the PC.
2. Wait 10 seconds and then re-insert the USB cable to the PC.
3. Navigate to InstaCal icon on your desktop and double click
4. Highlight the board number in the detection box and click OK
5. Right click on the board number in the InstaCal box and select configure.
6. Confirm settings are set to:
    a. 8 differential channels
    b. Factory calibration
    c. XAPCR Edge = Rising
    d. XAPCR Pin direction = input
    e. XDPCR Edge = Rising
    f. XDPCR pin direction = input
    g. ADC settling time = 10us
7. Click OK
8. Right click on the board number in the InstaCal box and select Calibrate – A/D
9. Click Calibrate
10. Click OK and Exit program.

10.22 Running a Report

ViViTest reports capture “a moment in time” during a valve’s trial. Results displayed on the report enable the user to identify the trial, valve and selected value used for the trial. A screenshot of the Process Display area is provided for visualization of the waveforms.

Using the “Print” icon a report is created within an HTML document. These files are automatically saved to your Files folder within the software directory.
NOTE: The report will open immediately to screen. A copy will have automatically been saved to the Files folder.

To Open and Print:

To keep a physical copy of the report users can:

1. Access the file via “Explorer” under C:\Program_Files\ViVitro_Labs\ViViTest\Files.
    NOTE: The file will be identified with .html format. The file name will be the identifier information entered for the valve and the date and time.

Open the desired report.

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NOTE: This will open in a Browser or the computers preferred .html file viewing program. Select to print from the menu.

10.23 Taking a Snapshot

Snapshots enable the user to display represented graphs of the trial's valve cycle activity waveforms at any moment.

Click the "Snapshot" icon to produce a .JPG file of the Process Display area. Extra data is included in the file in the top right corner of the graph to identify trial, valve and other related data.
Customizing the Snapshot:

The Process Display area can be manipulated to zoom in via toggling of the tool options within the graph.

To Save, Open and Print:

1. Selecting the Snapshot button will capture the Process Display area as it is seen on the screen. A save window will display to enable the user to customize the file name, alternately the user can opt to use the automatically assigned file name created from valve identifier information, date and time.
   NOTE: The Snapshot will not open immediately but be saved to the Files directory of the software for later viewing.
2. Access the file via "Explorer" under C:\Program_Files\ViVitro_Labs\ViViTest\Files.
   NOTE: The file will be identified with .JPG format. The file name will be the identifier information entered for the valve and the date and time or the customized name the operator assigned.
3. Open the desired Snapshot.

4. Select print from the menu.

10.24 Exporting CSV file data

For researchers/developers wishing to extract the data to create algorithms and calculations to provide specific results the option of producing a "raw data" .csv file is available. Users can access this under the Analyze tab either as "raw data" or by opening a "results table" and exporting the calculated data.
NOTE: The output presents as many cycles as displayed within the Process Display area.

By clicking on the Export CSV icon you can create a .CSV file once data has been accumulated.
The output is suitable to be viewed and manipulated within a .CSV file reading program. Data supplied includes trial and valve information as well as:

- Associated data creating the current trial scene;
- Raw collected Inflow/Outflow pressure measurements throughout the displayed cycles.

To Save, Open and Print:

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1. Selecting the Export CSV button will capture the data associated with the Process Display area as it is seen on the screen. A save window will display to enable the user to customize the file name, alternately the user can opt to use the automatically assigned file name created from valve identifier information, data and time.
   NOTE: The .CSV file will not open immediately but be saved to the Files directory of the software for later viewing.
2. Access the file via "Explorer" under C:\Program_Files\ViVitro_Labs\ViViTest\Files.
   NOTE: The file will be identified with .CSV format. The file name will be the identifier information entered for the valve and the date and time or the customized name the operator assigned.
3. Open the desired .CSV.
   NOTE: This will open within the computers preferred .CSV file viewing program.
4. Select print from the menu.

10.25 .ANA file

During the Analyze Process an .ANA file is created to store the save data collected. The .ANA file enables the operator to open the analysis information to allow comparisons.
To Save, Open and Print:

1. Using the Save button save the current data to an .ANA file. A save window will display to enable the user to customize the file name, alternately the user can opt to use the automatically assigned file name created from valve identifier information, data and time.

To retrieve the .ANA file select the desired analysis files on the bottom right side of the software under the directory C:\Program_Files\ViVitro_Labs\ViViTest\Files.
NOTE: This action will enable the operator to carry out print, snapshot and excel export activities pertaining to the data saved.

10.26 Log File

As an ongoing background feature of ViViTest, log files are created each time a user instructs the software to do an activity. These files can be accessed via the C:\Program_Files\ViVitro_Labs\ViViTest\Logs directory as a .LOG file.
To Open and Print:

1. Access the file via "Explorer" under C:\Program_Files\ViVitro_Labs\ViViTest\Logs.
   NOTE: The file will be identified with a .LOG format. The file name will be the identifier information entered for the valve and the date and time.
2. Open the desired .LOG.
   NOTE: This will open within the computers preferred .LOG file viewing program.
3. Select print from the menu.

10.27 Waveform Design

Users can change waveform designs by clicking on and selecting Edit Pump Waveforms.

Designing Custom Waveforms:

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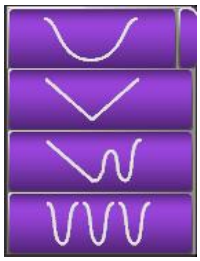
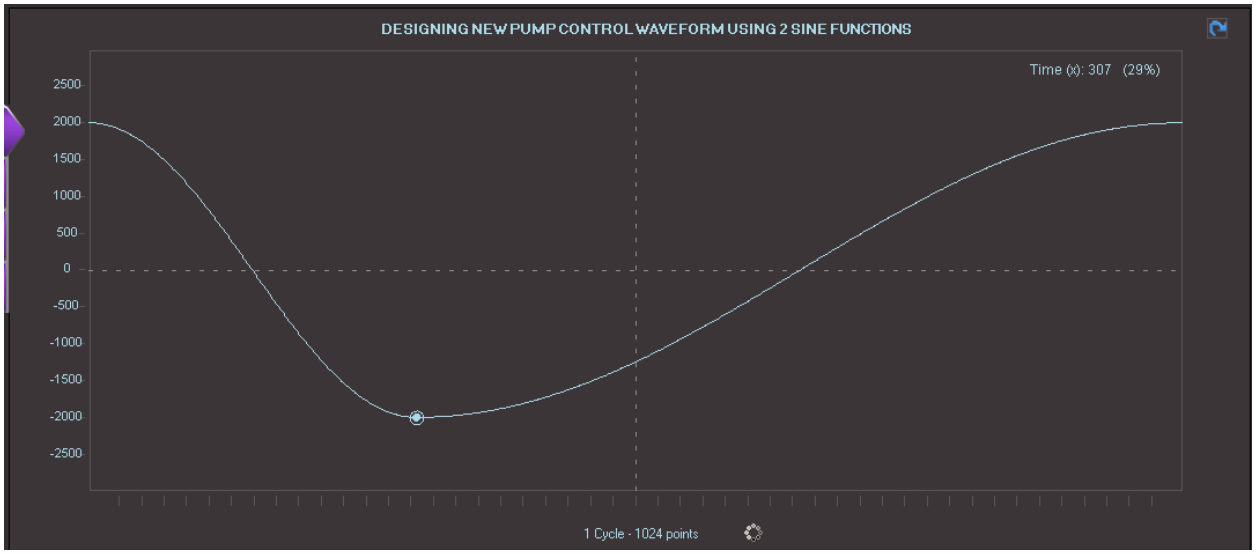


Waveforms

Users with special requirements may be interested in designing new custom waveforms. These 4 waveform buttons allow users to load and modify a dual-sine waveform, a 2-line waveform, a spline waveform and a wavetrain. After a custom waveform is designed it is then saved as a .WVF or .WVT file.

Users create a custom sine or 2-line waveform by dragging the bottom point of the 2-line V or dual sine valley in the X direction until the desired shape is achieved.



ViViTest – Waveform customization custom sine wave

Spline waveforms are created by dragging one or more of the spline points to create a new shape. New spline points are created by clicking on the line whereas points can be deleted by right clicking on the point. Users select a point along a segment and then drag that point up or down in the Y direction, until the desired shape is achieved.

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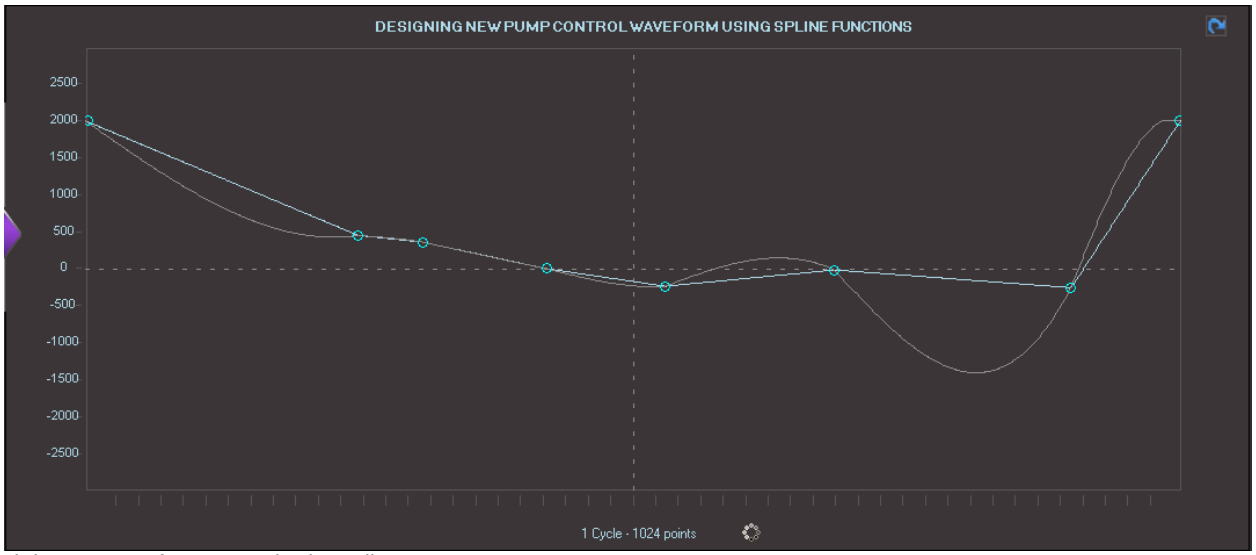
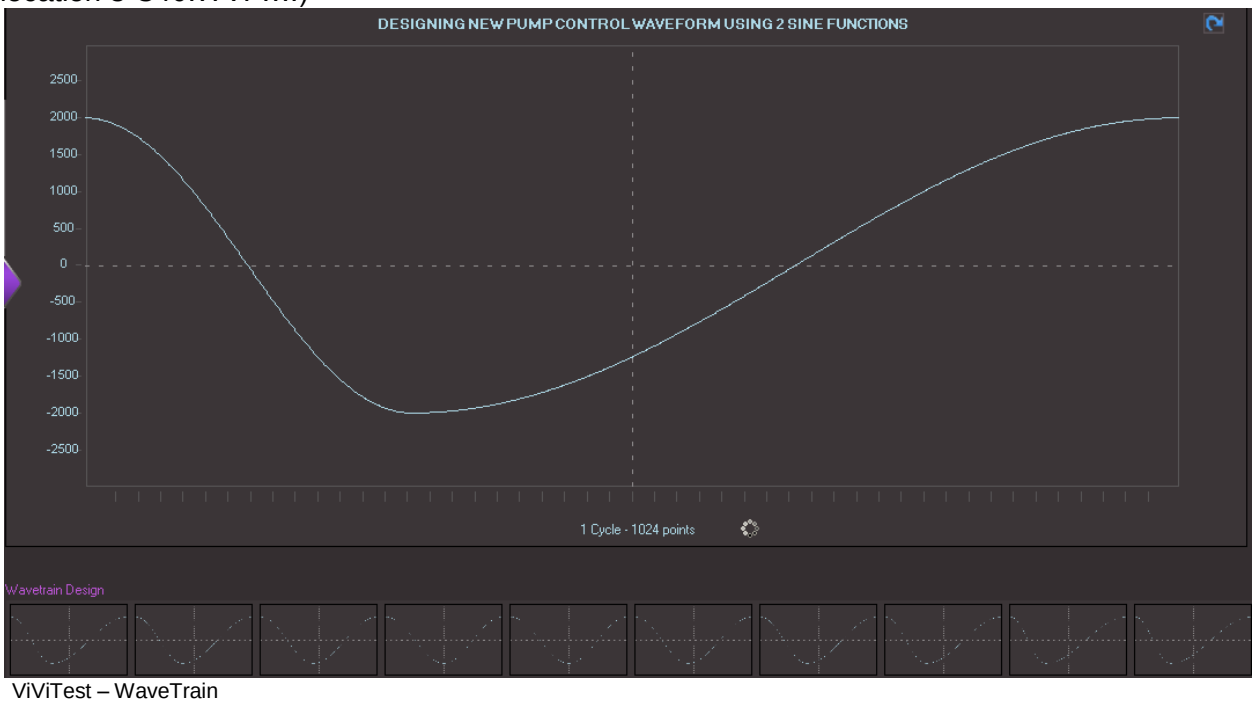


ViViTest – Waveform customization spline wave

Wavetrains are created by selecting one of the saved waveform from the waveform design files on the right hand side. Once the file has been selected, click on the first box (or desired location) in the Wavetrain Design window below the graph. Users can create a train consisting of a variety of waveforms up to 10 in sequence. (i.e. location 1-S30.WVF, location 2-S35.WVF, location 3-S40.WVF….)



ViViTest – WaveTrain

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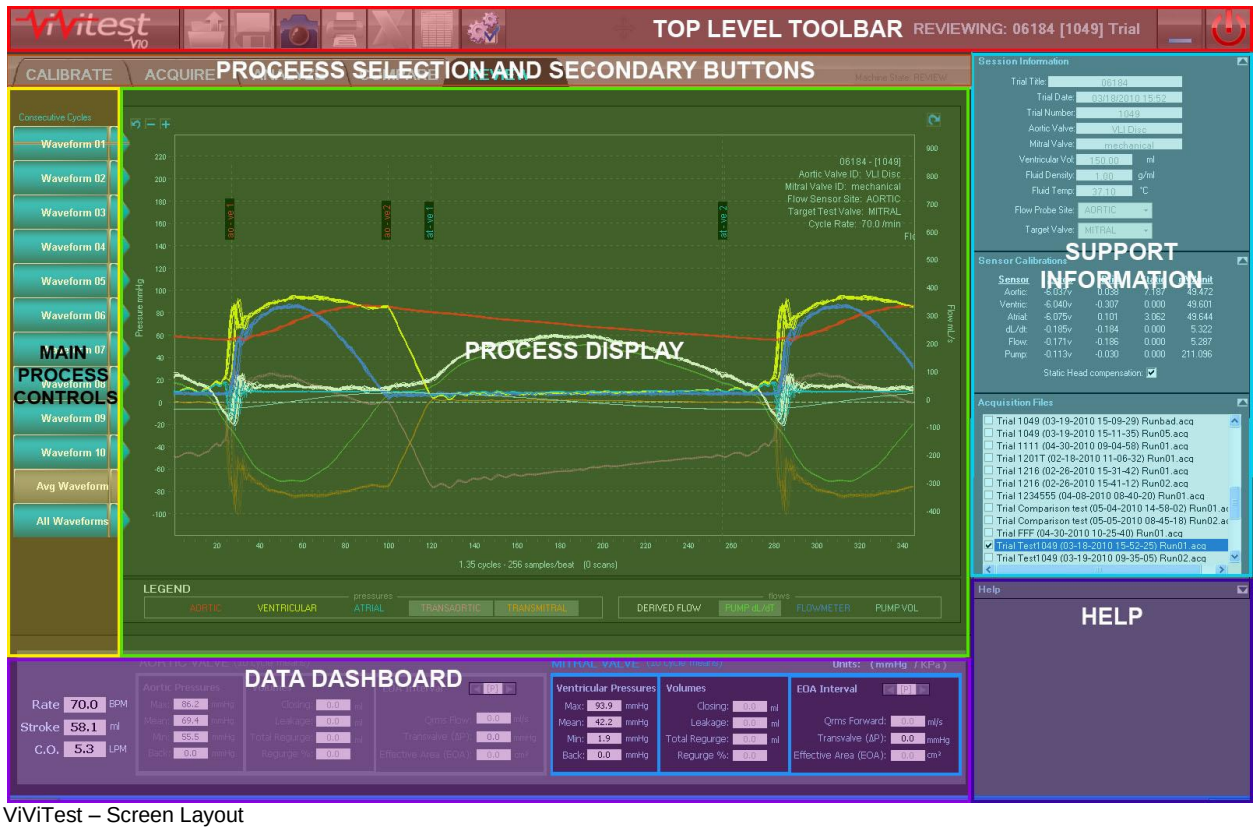
11. Software Interface Orientation



ViViTest – Screen Layout

11.1 Top Level Toolbar

Standard controls in the top level toolbar are:

Open File — Open an Acquisition (.ACQ) file for analysis or Analysis (.ANA) file for comparison graphing.

Save File — Used to save an Acquisition file when in Acquisition Process.

Snapshot — Captures a (.JPG) screen snapshot of the user interface. (See Tooltip for details)

Print — In Analysis or Compare processes users can produce a report (.HTML) of results which can then be viewed and printed in any browser.

Export CSV — Save Acquisition or Compare data to a comma separated value (CSV) file for further processing.

Data Table — In Analysis process the 10 sequential cycles saved to an .ACQ file are analyzed and the resulting means data shown in tabular format.

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	Options Menu	Access to all software features and information about ViViTest.
	Minimize	Minimizes the software to the windows toolbar to enable the user to carry out other activities on the computer screen.
	Exit	Closes the application.

NOTE: Most controls on the user interface have tooltips that give a brief explanation of usage whenever the mouse is hovered over the control.

NOTE: All files saved from the software are saved to the Files folder of the software directory. (e.g. C:\Program Files\ViVitro Labs\ViViTest\Files)

11.2 Process Selection and Secondary Buttons

These tabs transfer you to the various components of the software.
These are:

- Calibration Process,
- Acquisition Process,
- Analysis Process,
- Compare Process and
- Acquisition Review.

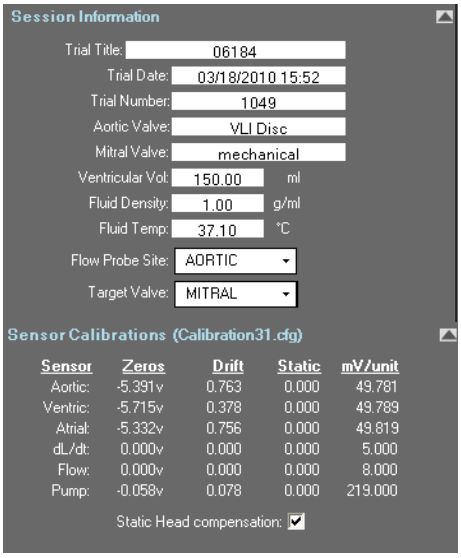
11.3 Support Information Area

- Demographic statistics, process table data and context help in collapsible panels at screen right.
- Displays editable and non-editable information relating to the Process Display area.

Collapses the selected data area.
Opens the selected data area.

Session Information

Set up key valve identifier information for each time a new valve is being tested. Ensure that the correct flow probe site is selected to gain correct readings. Data entered within this area will be used to create automated file names.

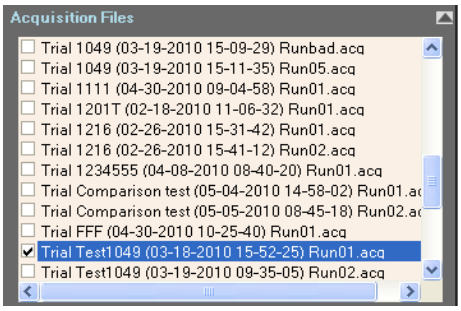

Session Information
Trial Title: 06184
Trial Date: 03/18/2010 15:52
Trial Number: 1049
Aortic Valve: VLI Disc
Mitral Valve: mechanical
Ventricular Vol: 150.00 ml
Fluid Density: 1.00 g/ml
Fluid Temp: 37.10 °C
Flow Probe Site: AORTIC
Target Valve: MITRAL
Sensor Calibrations (Calibration31.cfg)
Sensor Zeros Drift Static mV/unit
Aortic: -5.391v 0.763 0.000 49.781
Ventric: -5.715v 0.378 0.000 49.789
Atrial: -5.332v 0.756 0.000 49.819
dL/dt: 0.000v 0.000 0.000 5.000
Flow: 0.000v 0.000 0.000 8.000
Pump: -0.058v 0.078 0.000 219.000
Static Head compensation:

Sensor Calibrations

Represents the collected calibration values within the selected calibration file.
Enable/disable Static Head compensation to alter readings as desired.

Acquisition Files

Allows access to all .ACQ files saved for previous valve trials.



Analog Voltage Information

Provides analog voltage information for the flow probe and transducers used within the system.

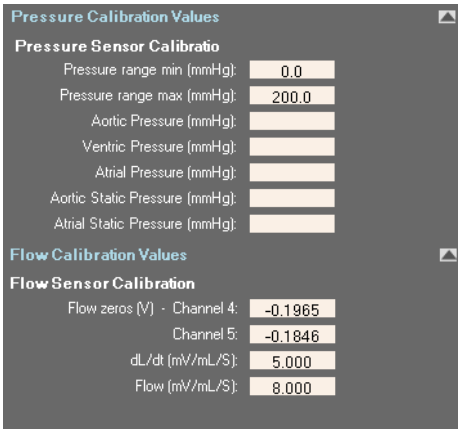
Analog Voltage Information
Chn Volts Std Dev
Aortic 1: - 5.371 0.002
Ventric 2: - 5.694 0.002
Atrial 3: - 5.311 0.003
dL/dt 4: - 0.181 0.002
Flow 5: - 0.058 0.001
Pump 6: - 0.058 0.002

Pressure Calibration Values

As pressure calibration is carried out the values are represented within this area for reference.

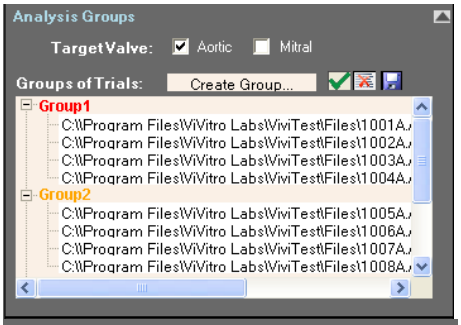
Flow Calibration Values

Provides the flow probe calibration values for reference.



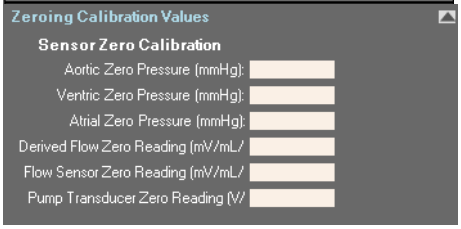
Analysis Groups

Allows the user to create groups of valve trial data for analysis and comparison.
Users have the ability to select .ANA files from the list below to assign them to a group.
Groups can be cleared and created easily within this area and are saved as .GRP files.



Zeroing Calibration Values

Provides pressures, flow and pump zeroing calibration values for reference.


Zeroing Calibration Values
Sensor Zero Calibration
Aortic Zero Pressure (mmHg):
Ventric Zero Pressure (mmHg):
Atrial Zero Pressure (mmHg):
Derived Flow Zero Reading (mV/mL/
Flow Sensor Zero Reading (mV/mL/
Pump Transducer Zero Reading (V/

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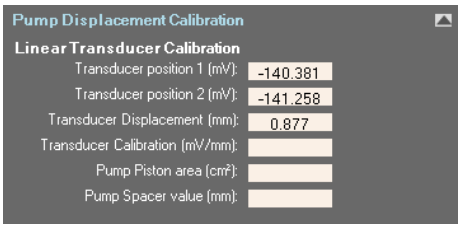
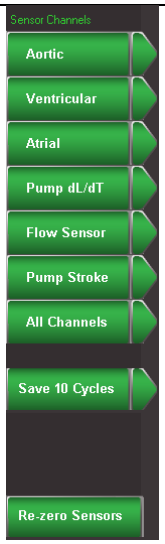
Pump Displacement Calibration
Displays pump linear transducer calibration values as input in the Wizard and represented within the .CFG file.

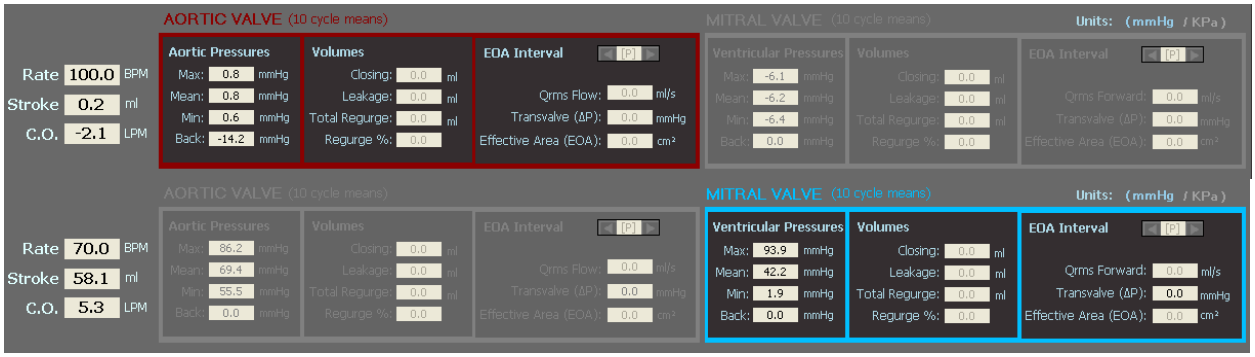


11.4 Main Process Controls

Control buttons which modify or add attributes to the Process Display area.

Calibrate	Acquire	Analyze	Compare	Review
				

11.5 Data Dashboard

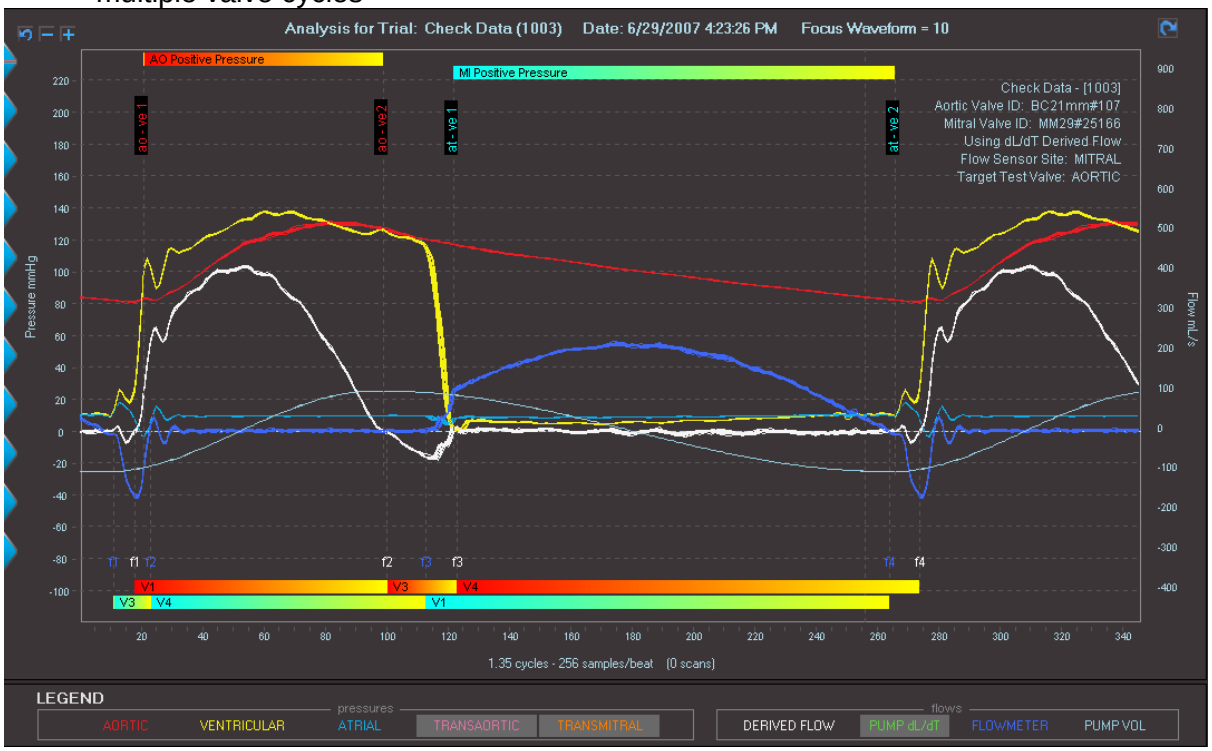


- Information relating to Aortic or Mitral Valve measurements.
- Provides non-editable summary data for both the aortic and mitral valves,
- Measurement units can be toggled by using the mmHg / KPa button.

11.6 Process Display

Displays the activity of information gathered. In most cases the following will be displayed:

- a multi-axis graph typically showing both pressure and flow data over a 1.35 pump cycle
- most recent pressure sensor values for most recent ADC analysis
- multiple valve cycles



- A legend bar indicating the meaning of the various coloured graph traces. These can be turned on/off to highlight specific data to be viewed:
  - RED line – Aortic Pressure
  - YELLOW line – Ventricular Pressure
  - BLUE line – Atrial Pressure
  - PINK line – Transaortic Pressure
  - ORANGE line – Transmitral Pressure
  - WHITE line – Derived Flow
  - GREEN line – Pump dL/dt
  - DARK BLUE line – Flow meter
  - GREY line – Pump Volume

The graph can be manipulated by:

Refresh – this allows the data to be refreshed/reloaded to display the latest data.

Restore – enables the changes done to the graph view to revert to 1:1.
Increase/Decrease (zoom) – manipulates the interval frequency of the measured pressure to bring the graph closer or further away.
Up/Down – enables the user to position the adapted graph data more centrally on the screen by adjusting the Y axis.

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11.7 Process Output

The software uses a number of various calculations to output accurate and relevant information for your review. Some of the standard formulas have been listed below for your ease of reference. Please reference Appendix E for a more complete list of calculations used. For anything further please contact ViVitro Labs directly.

$$\mathrm{EOA} = \frac{Q_{rms}}{51.6\sqrt{\frac{\Delta p}{\rho}}}$$

$$Q_{rms} = \sqrt{\frac{\int_{t1}^{t2} Q(t)^2\, dt}{t_2 - t_1}}$$

11.8 Help

Information pertaining to the screen or the software in general Help is available throughout the use of ViViTest.

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12. System Maintenance

12.1 Required Fluid

There are 3 zones of your system that will require differing fluid requirements.

1. The main operating zone: within the Model Left Heart, Peripheral Resistance Controller and connecting tubes.
2. The pulse duplicating zone: within the pump head, VIA (if installed) and Ventricle Chamber.
3. The heat exchanging zone: within the heat exchanger, heat bath, heat pump and tubing.

Zone 1
Depending on your requirements the following fluid information is provided as a guide for use in the system.
Fluid:

- Saline; or
- A blood analogue fluid consisting of ~35% glycerin 65% saline by volume. This mixture having a density of approximately 1.133 g/ml at 20°C will have a viscosity of approximately 3.2 mPa·s at 37°C. This approximates the viscosity of blood.
- Any solution used must be mixed to 0.9% NaCl concentration to operate the electromagnetic flow probes properly.

Volume: Approximately 2 liters

Zone 2
Fluid: Distilled water mixed with an appropriate biocide may be required to reduce the bioburden impact of contamination. Contact a supplier for recommendations.

Zone 3
Fluid: Water mixed with an algaecide to prevent algae growth.
NOTE: The fluid surrounding the ventricle in the hydraulic chamber and the VIA fluid is normally distilled water and can remain in situ for a period of time. It should however be replaced when bio-film contamination compromises visibility. In general, replace the hydraulic fluid every 2 months. If the system is not used for extended periods, the hydraulic chamber fluid should be drained and all components cleaned with a soft cotton cloth and detergent and then dried before storage.

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12.2 Draining Fluid

NOTE: Prior to draining the system, ensure the flow meter is turned off. Operatinig the flow meter without fluid in the system will damage the flow probe.

To change valves, or to disassemble the system, the test fluid must first be drained. To do this, release the drain tube clamp and collect fluid in a container of ~2 liters volume. Re-clamp drain after system is drained. Fluid remaining in the left atrium reservoir can be drained by temporarily disconnecting the green colour coded hose connected to the left atrium port. Remaining test fluid in the aortic root above the aortic valve can be removed by syringe through a pressure port stopcock.

Replacing frequency:

Zone 1: Will be replaced by natural course due to the changing of prosthetic structures on a regular basis.

Zone 2: Replace every 2 months.

Zone 3: Replace every 2 months.

Instructions Zone 1:

1. Ensure the system is turned off, including the flow probe/s
2. Place bucket under Ventricle Chamber.
3. Open drain valve situated beneath the chamber.
4. Allow to completely empty.

NOTE: Ideally you would allow it to completely dry before placing further fluids in the system.

Instructions Zone 2:

1. Ensure the system is turned off, including the flow probe/s
2. Place bucket under Ventricle Chamber
3. Open luer drain port on bottom of Ventricle Chamber.
4. Tip the entire system to 30º with the motor at the higher end. (This will aid the draining of the VIA and pump head).
5. Return to horizontal position.

NOTE: Ideally you would allow it to completely dry before placing further fluids in the system.

Instructions Zone 3:

Ensure the system is turned off, including the flow probe/s. Drain as per the Heat Bath manufacturers recommendations.

12.3 Cleaning Acrylic Fluid

The other acrylic plastic parts can be cleaned using a soft cotton cloth with detergent and water. If required, acrylic parts can also be sterilized by using glutaraldehyde or a dilute hydrogen peroxide solution. Keep these solutions away from the flow probe electrodes. Alcohol, abrasives and solvents should never be allowed to come into contact with the acrylic components of the Model Heart.

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12.4 Aorta Replacement

CAUTION: THE GLASS AORTA IS FRAGILE - HANDLE WITH CARE - DO NOT APPLY UNDUE STRESS

1. Remove the original aorta/exit tube assembly from the load assembly:
    - remove two 2-56 bolts on the clamp retaining the catheter to the side of the aorta exit tube;
    - remove four 6-32 x 3/8 inch bolts to detach the white plastic plate at the entrance of the glass aorta;
    - loosen the two 6-32 bolts and rotate the washers near the exit end of the glass aorta to allow aorta assembly removal;
    - push the exit end of the plastic aorta exit tube to remove glass aorta and exit tube assembly.
2. Remove remnants of the original aorta and silicone sealant from the exit tube.
3. Attach three 2 cm strips of adhesive tape equally spaced on the inside surface of the new aorta exit with 1 cm of tape projecting above the end.
4. Hold the aorta exit tube above the glass aorta so that the pressure port is aligned with a sinus edge.
5. Mate and center the exit tube counter bore with the aorta and then use the tape to hold the two parts together.
6. Use a syringe filled with silicone sealant with attached dispensing tip (e.g. EFD Inc. #5118PPS-B) to fill the gap between the glass and aorta exit tube taking care not to entrap air bubbles which could cause leaks. Any excess sealant should be wiped before curing occurs.
7. Let sealant cure (24 hours).
8. Remove adhesive tape from inside surfaces of assembly.
9. Re-insert assembly into housing reversing the 4 bulleted steps shown above in step 1.

12.5 Ventricle Member Replacement

The model ventricle and bonded drain tube are made of silicone rubber and should have a long flex life. If damaged, they may be repairable using RTV Silicone rubber. Otherwise, replace as follows:

1. Remove 8 X-head bolts and valve block retaining tabs then remove block
2. Remove polycarbonate base filler ring
3. Move ventricle base aside to gain access to the bonded silicone tube and pull off from polycarbonate tube**.**

Reverse above sequence to install new ventricle.

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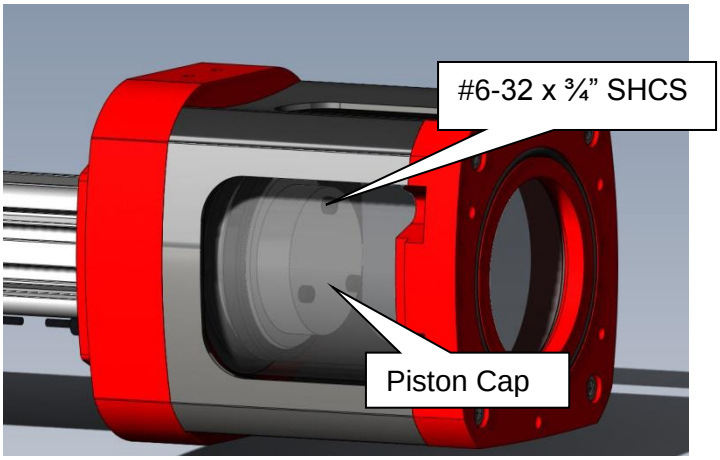
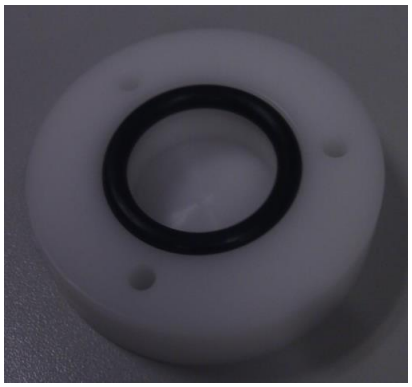
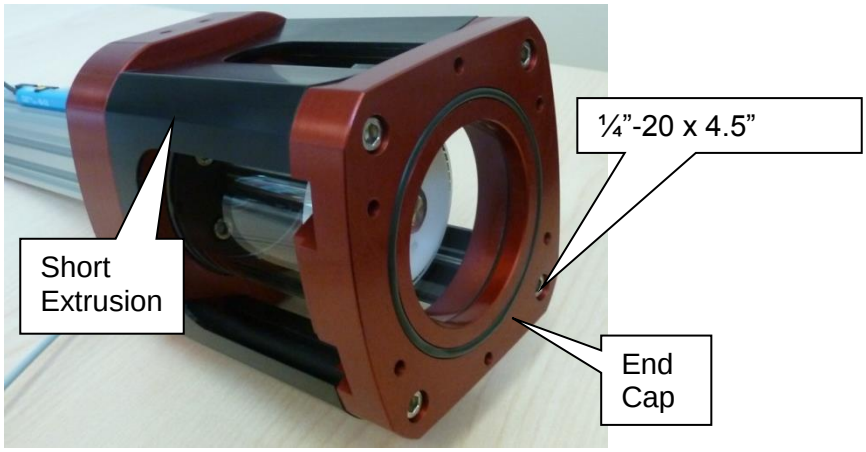
12.6 Piston Seal Replacement

1. Remove the three #6-32 x ¾" screws located on the front of the piston and remove the SuperPump Piston Cap (09533).

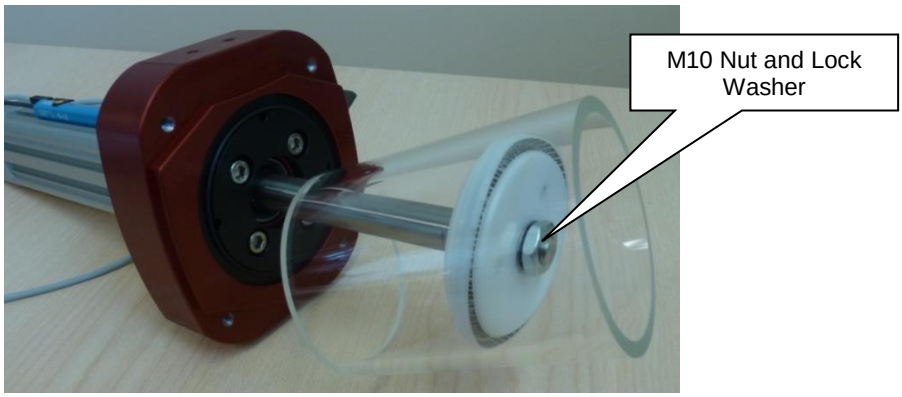




2. Remove the four ¼"-20 x 4.5" screws on the front of the pump head.



3. Remove the Short Extrusion (08981) and end cap from the pump assembly.



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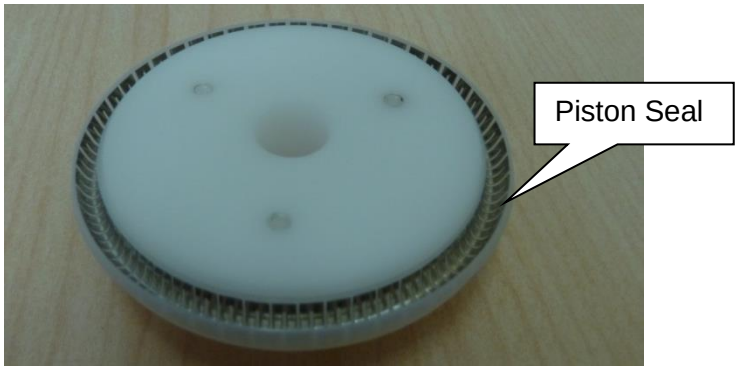
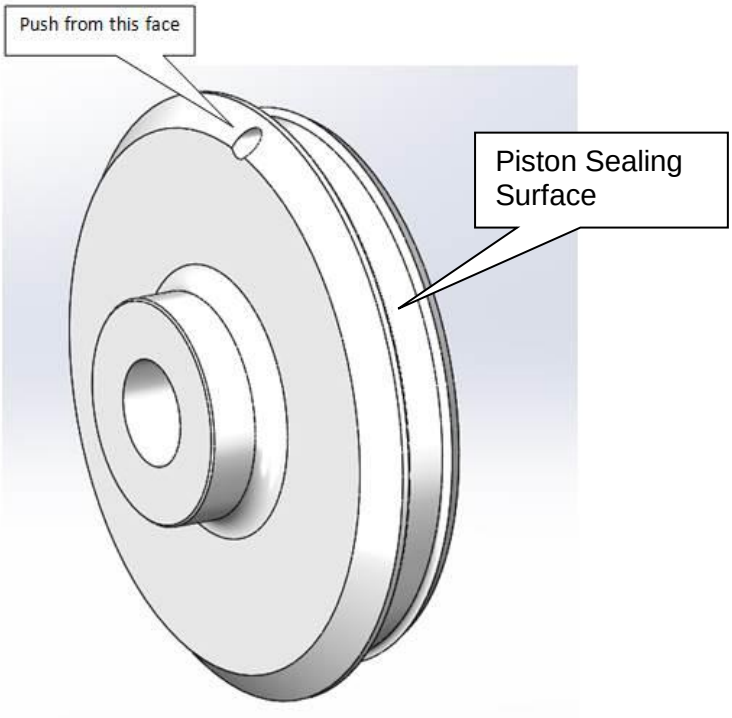
4. Carefully remove the glass cylinder from the piston.
   CAUTION: DURING THIS STEP BE CAREFUL WHEN HANDLING THE GLASS CYLINDER AS THE EDGES MAY BE SHARP.
5. Remove the M10 nut and lockwasher from the front of the piston.
6. Remove the SuperPump Piston (09532) from the rod end.



7. Locate the small hole near the outer edge of the piston. Holding the piston firmly, press off the piston seal with a small hex key. Be sure not to scratch or damage the piston sealing surface during this step.



8. Check the sealing surface of the piston for scratches that are either visible or can be felt with a fingernail. Ensuring the orientation is correct, snap the piston seal onto the piston carefully by hand.
9. Clean any dust and debris from the glass cylinder. Apply two pieces of electrical tape to one edge of the glass cylinder.

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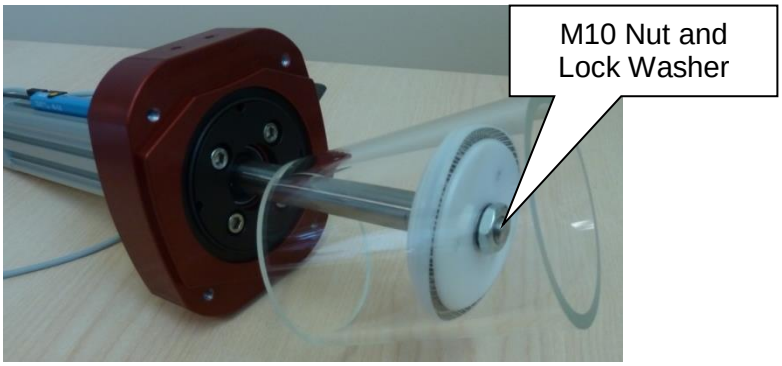
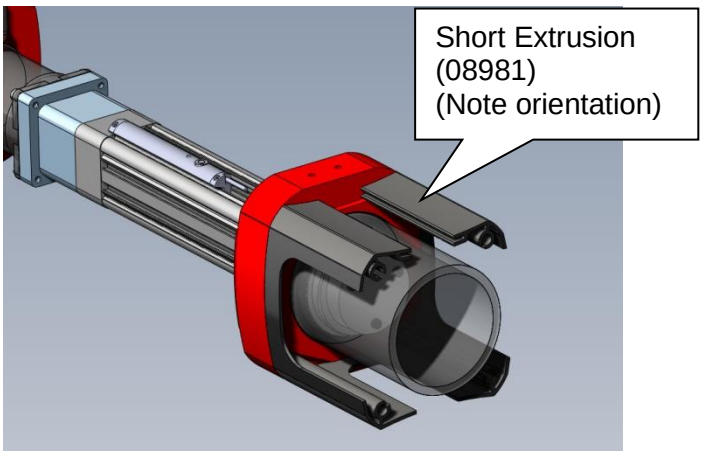
10. Push the piston into the glass cylinder (SPM042) using the tape as a glide point. Remove the electrical tape from the Glass Cylinder (SPM042).
11. Slide rod end into the SuperPump Piston (09532). Fasten in place with a M10 Lock Washer (09538), Loctite, and the nut from the rod end. Ensure the glass cylinder does not bump against the cylinder rod.



12. Push the Glass Cylinder (SPM042) onto the pump. Ensure that the piston does not come close to the sharp edge of the glass cylinder.
13. Place the Short Extrusion (08981) onto the pump. The gap in the short extrusion should be in line with the longer flat section of the central stand.



14. Push O-Ring-235 (SPM072) into the groove on the outside face of End Cap (08975).
15. Push O-Ring-335 (10084) into the groove on the inside face of End Cap (08975).

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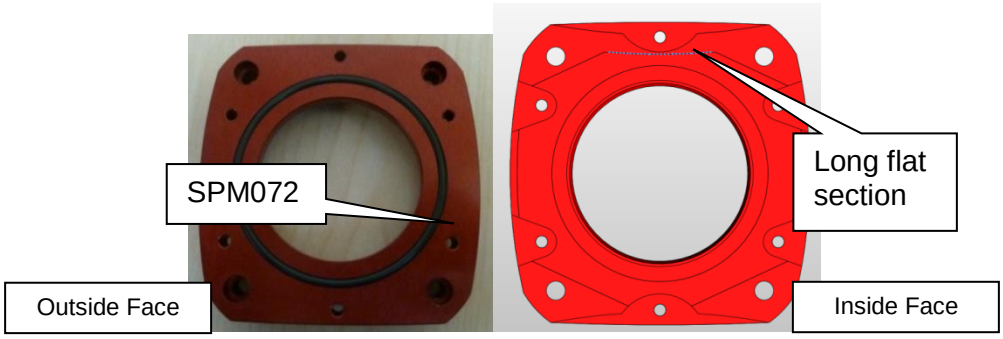
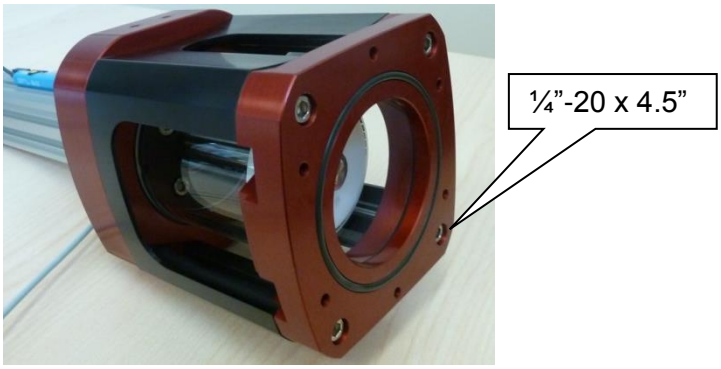


16. Fasten the End Cap (08975) onto the pump head with four ¼”-20 x 4.5” SHCS (08980). Take care not to chip the edge of the glass with the aluminum parts. Tighten the fasteners gradually to prevent uneven stress on the glass cylinder. Compress the sides of the Short Extrusion (08981) while tightening so that the sides sit flush with the end cap faces.



17. Check for damage on O-Ring (09534) into the recess of the SuperPump Piston Cap (09533).
18. Fasten the SuperPump Piston Cap (09533) to the SuperPump Piston with three #6-32 x ¾” SHCS (HCM079). Loctite is not required.

The SuperPump is now ready for installation of the Viscoelastic Impedance Adaptor (VIA) or Model Left Heart.

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13. Appendix A – Supplemental Component Notes

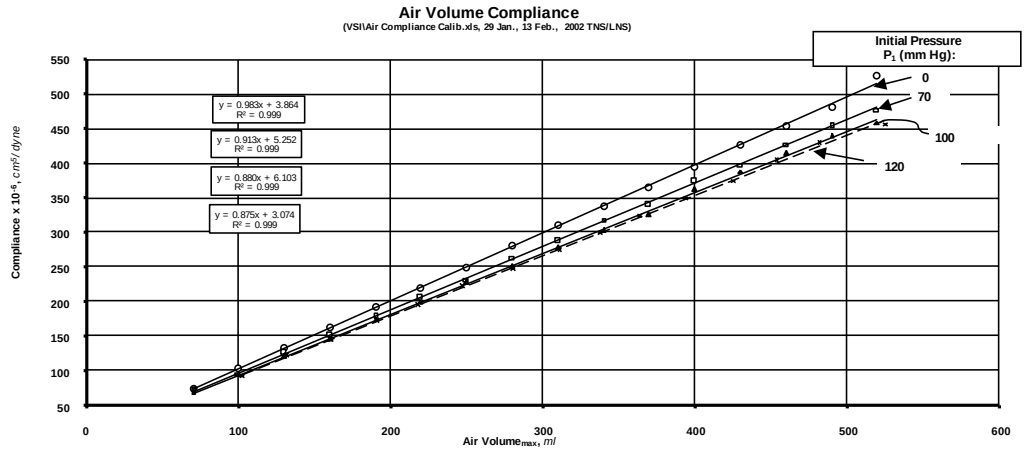
The extra compliance available with Large Volume Compliance bottle is useful in applications where aortic root or LVOT compliance is minimal. This will occur in applications where low compliance conduits are being utilized. This is typical when stentless aortic valves, silicone rubber aortas (PVL applications) and LVOT models are involved in the test work. If desired, the air volume simulating characteristic compliance can be reduced by allowing the fluid level to rise higher in the characteristic compliance chamber and/or by adding water to the supplementary bottle as shown below for the optional large supplementary compliance bottle.
The diagram depicts how compliance adjustment is accomplished using water to reduce the air volume.
Note on compliance modeling
Tissue compliance is simulated in the Model Heart and VIA models by using contained air volumes. These air volumes are adjustable and cover a physiologic range for simulating left ventricle, aortic root and systemic arterial compliance. Compliance is defined as the ratio of volume change to pressure difference as follows:

$$\text{Compliance} = \frac{\Delta V}{\Delta P \bullet 1333.2} \ \text{cm}^5/\text{Dyne}$$

Where:

ΔV = change in contained air volume in ml
ΔP = pressure difference (mm Hg) caused by volume change ΔV
$\Delta P = P_2 - P_1$
P_1 = initial static pressure in mmHg
P_2 = final static pressure in mmHg
Conversion factor: 1 mmHg = 1333.2 Dynes/cm²

The following graph shows experimental data for contained air volumes versus compliance. Initial static pressure $_1$P ranged from 0 to 120 mm Hg. Best fit linear equations are given for the four data lines. It can be seen that in a contained air volume, as initial pressure condition increases, compliance decreases.
Air volume max values that were experimentally found appropriate for simulating left ventricle, aortic root and systemic arterial compliance are:
Left ventricle -Source compliance air volume = 120 ml; -output compliance air volume = 50 ml
Aortic root = 640 ml
Systemic arterial = 615 ml
These air volume compliances helped provide realistic pressure and flow wave forms under pulsatile flow conditions. Air volumes less than these can simulate various patho-physiologic or stiffened tissue conditions where tissues may exhibit less compliance.



14. Appendix B – Installing LVOT Accessories (Optional)

The ViVitro Labs Inc. Pulse Duplicator System and aortic system has been used by some clients to test stentless aortic valves and simulate left ventricular outflow tracts (LVOT). Although compliant aortic roots with sinuses are not currently provided by ViVitro Labs Inc., this instruction describes a method whereby optional ViVitro Labs Inc. flanges may assist in mounting the researcher's own structures into the ViVitro Labs Inc. Pulse Duplicator System.

NOTE: The diameter of a compliant aorta will change under dynamic flow and pressure conditions. The minimum diameter of the aorta during pulsatile flow testing should be approximately that of the relaxed or molded diameter of the aorta. This can be achieved by adjusting the mean pressure on the outside of the aorta using a syringe to inject or withdraw air from the aortic root compliance chamber.

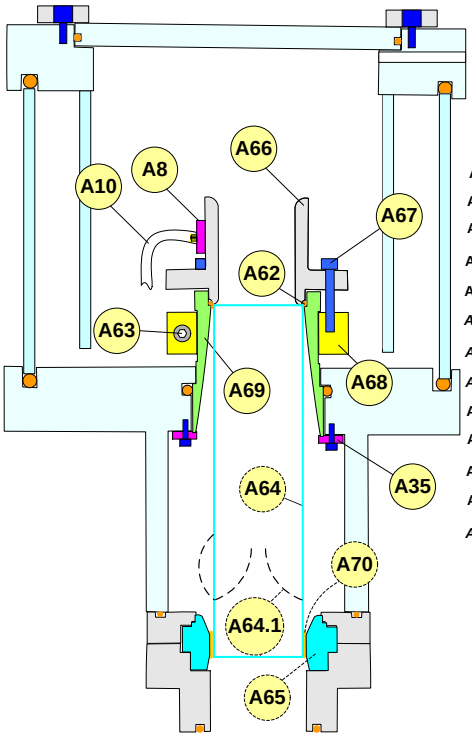


FIGURE LEGEND:

A8 Catheter clamp (with V-006 O-ring) & 2-56 x 3/8 inch s.s. bolts, 2 ea.
A10 High pressure catheter tube
A35 Aorta clamp washers & 6-32 x 3/8 inch s.s. bolts, 2 ea.
A62 Silicone O-ring (shown bonded to compliant aorta exit):
A63 Clamping bolt (for part #8), 6-32x¾ inch, 1 each
A64 *Compliant silicone aorta/RVOT no sinuses (13-28 mm ID)*
A64.1 *Stentless Aortic valve mounted to aorta (user supplied)*
A65 *Silicone rubber inlet ring (bonded to silicone aorta/RVOT)*
A66 Outlet tube (male) for Flex aorta:
A67 Clamping bolts 6-32 x ¾ inch, 3 each
A68 Outlet tube clamp for Flex aorta, (common for all sizes)
A69 Outlet tube (female) for Flex aorta:
A70 Bond between aorta and aortic inlet ring

NOTE: It is recommended that these structures be made from silicone rubber. A silicone rubber aorta will bond easily to the other silicone rubber components that are needed using a silicone rubber sealant.

1. Custom make the aortic inlet ring from silicone rubber following procedures given in this manual.
2. The aortic ring is then bonded to the inflow of the aorta using Dow Corning flowable silicone sealant #734.

NOTE: A silicone rubber O-ring is supplied and is bonded to the outlet of the compliant aorta with the same sealant.

NOTE: The grey and green tubes are supplied in various ID sizes with internal diameters (13-28mm)

NOTE: Appropriate sized silicone rubber O-rings are also supplied for bonding to the exit end of the aorta.

3. Before mounting the compliant aorta/stentless valve and accessories, remove the standard glass aorta assembly following the instruction in the operating manual for aorta replacement.

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4. Bond a silicone O-ring {A62} to the exit end of the desired compliant aorta and mould a silicone rubber inlet ring {A65} with an inside diameter matching the inflow end of this aorta {A64}.
5. Select matched sized tubes {A66} and {A69} appropriate to the size of the aorta being attached.
6. With the clamp {A68} detached, insert the green tube {A69} into the aortic assembly housing and secure with the two clamping washers and bolts {A35}.
7. Place the outlet tube clamp {A68} over the green tube {A69}, temporarily leaving the bolt {A63} loose.
8. Attach the catheter {A10} to the grey outlet tube {A66} using the catheter clamp and bolts {A8}.
9. Mate the grey {A66} and green {A69} tubes and rotate the yellow clamp {A68} so that the three bolt {A67} holes align.
10. Tighten the clamp screw {A63}.
11. Insert the aorta exit O-ring {A62} between the grey {A66} and green {A69} tubes and clamp using the three supplied bolts.
12. Insert the inlet of the aorta into the silicone rubber inlet ring {A65} and before bonding the two components together, assure that there is no twisting or buckling of the aortic walls.
13. Once the bond is secure, remove the aorta from the assembly and proceed with mounting the stentless aortic valve into the compliant aorta.

NOTE: This process may involve suturing the valve the inside of the aorta. Resulting suture holes and sutures can be reinforced and sealed with a bead of silicone sealant on the inside and outside of the aorta. In this regard, silicone rubber sealant will vulcanize in water and therefore allows tissue type stentless valves to be kept moist.

14. With stentless valve and aorta assembly complete, install assembly into the Pulse Duplicator System aortic assembly housing and proceed with function testing

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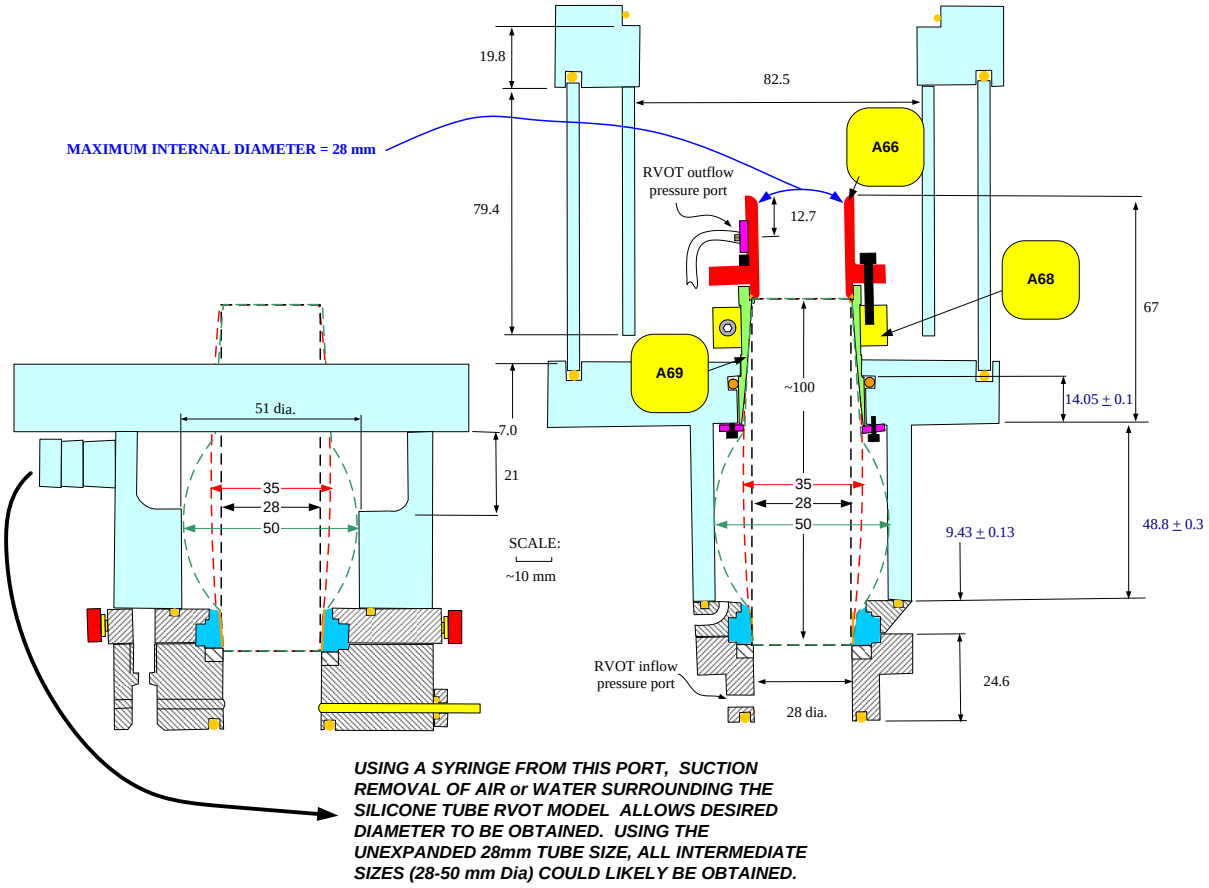
15. Appendix C – LVOT Simulation Assembly (Optional)

This sketch depicts a mounting scheme using silicone tubing to simulate a variety of LVOT sizes. Parts A66, A68 and A69 are custom fitted to accommodate silicone tubes with relaxed tube ID's of 13, 16, 19, 22, 25, and 28 mm. The 28 mm tube may be expandable up to about 50 mm OD and cover intermediate sizes as indicated.



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16. Appendix D – Components and Parts Lists

16.1 SuperPump

1 SuperPump (includes pump head and motor pre-installed)
1 SuperPump Motor power supply cable
1 SuperPump Amplifier
1 SuperPump Amplifier power supply cable
1 Cooling fan
1 SuperPump mounting shelf

16.2 Model Left Heart

1 Ventricle chamber
1 Aortic chamber
1 Atrium chamber
1 Aortic standoff
1 Accessories kit (includes 2 compliance chambers, 1 peripheral resistance controller and 4 colour coded silicone tubes)
1 Accessories table
2 Mechanical disc valve

16.3 Data Acquisition System

1 I/O Module
1 Computer and associated components
1 ViViTest Software package
1 USB cable
3 Short BNC cables
3 Long BNC cables

16.4 Flow Measuring

1 Flow Measuring controller
1 Flow probe
1 Flow probe cable kit (includes cable to flow probe and ground)

16.5 Optional System Components

Heat Exchanger
1 Heat bath including pump and controller box
1 Heat Exchanger
2 Long silicone tubes
1 Short silicone tube
1 Power supply cable
Manometer

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17. Appendix E – Glossary

Test Fluid	Fluid used within the chambers, pump and pump head.
Aortic Chamber	Provides (in conjunction with the Aortic Root Compliance Chamber and Aortic Compliance chamber) characteristic resistance which simulates the left aortic functions of the heart.
Atrium Chamber	Provides characteristic resistance which simulates the left atrium functions of the heart.
Aortic Root Compliance Chamber	Provides (in conjunction with the Aortic Chamber and Aortic Compliance Chamber) characteristic resistance which simulates the left aortic functions of the heart.
Aortic Compliance Chamber	Provides (in conjunction with the Aortic Chamber and Aortic Root Compliance Chamber) characteristic resistance which simulates the left aortic functions of the heart.
Mean Atrial Pressure	Measurement derived from the pressure created between the Atrium Chamber and the Ventricle Chamber to simulate the left atrial pressure in the heart in the mitral heart valve region. Measurement is gained using a Mitral Flow Probe.
Aortic Pressure	Measurement derived from the pressure created between the Ventricle Chamber and the Aortic Standoff to simulate the left aortic pressure in the heart in the aortic heart valve region. Measurement is gained using an Aortic Flow Probe.
Ventricular Pressure	Pressure created in the Ventricle Chamber prior to the Aortic heart valve opening.
DL/DT	A signal provided by the SuperPump Amplifier indicating the pump's flow rate in liters per unit time. dL/dt pump flow data represents overall system flow and can be used in conjunction with the flow meter to derive the flow across the valve opposite to the flow meter.

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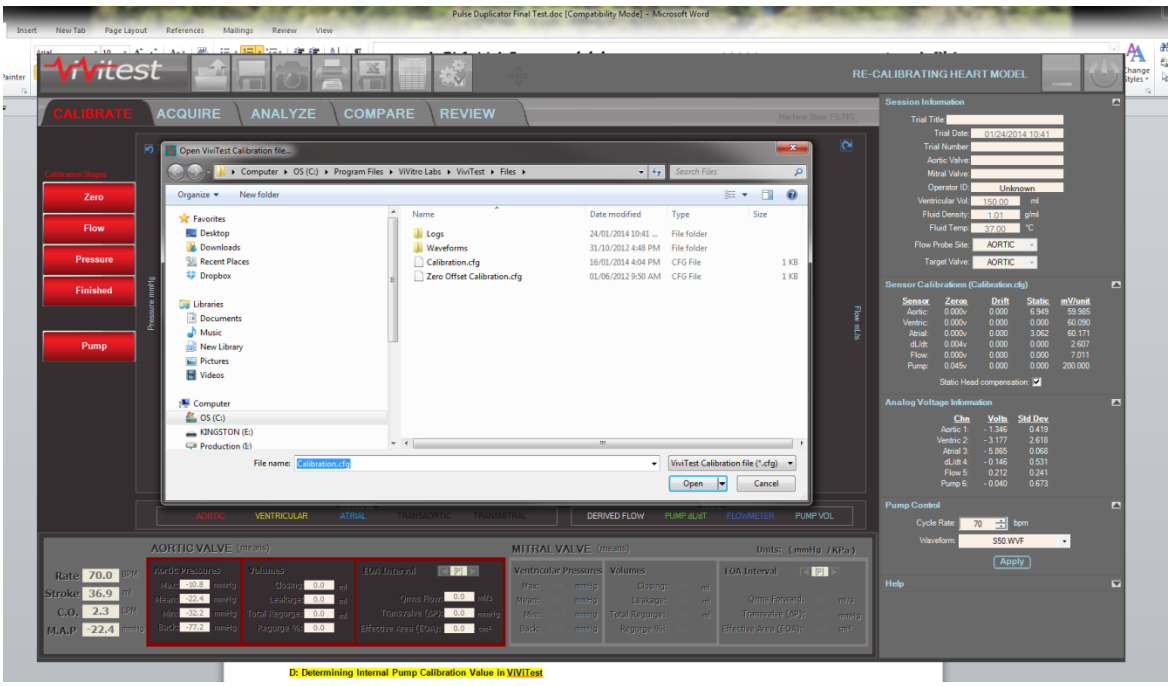
18. Appendix F – Pump Calibration Procedure

18.1 Resetting Values

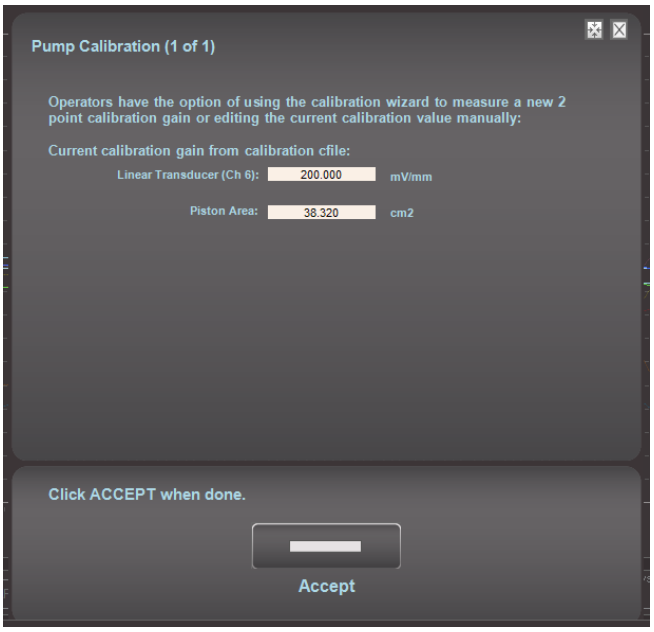
1. Run ViVitest Software. Select the Calibration Tab. Open the calibration file: Calibration.cfg.



D: Determining Internal Pump Calibration Value in ViViTest

2. Turn the amplitude to zero.
3. Select the Pump calibration tab.
4. Change the Linear Transducer (Ch 6): value to 200.000 mV/mm. Verify that the Piston Area is set to 38.32 cm2. Select Accept.

5. Select the Calibrate Tab. This saves changes to the calibration file Calibration.cfg. Close the print preview dialog box. Select the Calibrate Tab. Open the Calibration.cfg file. (this process saves the 200.000 mV/mm value).


Pump Calibration (1 of 1)
Operators have the option of using the calibration wizard to measure a new 2 point calibration gain or editing the current calibration value manually:
Current calibration gain from calibration cfile:
Linear Transducer (Ch 6): 200.000 mV/mm
Piston Area: 38.320 cm2
Click ACCEPT when done.
Accept

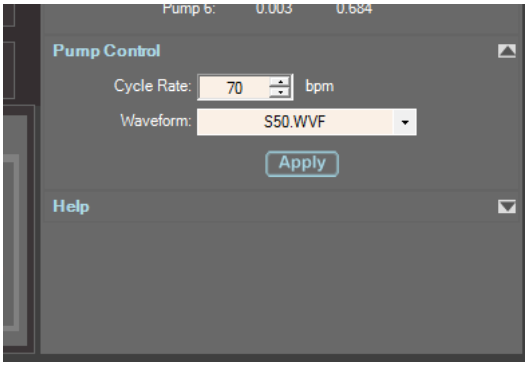
18.2 Determing Interal Pump Calibration Value

1. Turn the SuperPump Actuator on. Set the internal waveform to S50_70. Adjust the amplitude knob to an arbitrary value and watch to ensure ViViTest is showing a sine waveform for the Pump Vol signal. All other signals can be hidden if desired.
2. Turn the SuperPump amplitude knob up the values described in the table below. Record the controller stroke volume for both the controller display and the ViViTest display. Calculate the values for the right hand column. Average the results of the 3 calculations. (should be ~ 197 mL/stroke)

Controller Stroke (mL/s)	ViViTest Stroke (mL/s)	= (ViViTest value / Controller Value) * 200
50		
100		
150		
Average:		

18.3 External Gain Value

1. Ensure there is not enough water in the load assembly that when the pump is turned to 100% amplitude it sprays.
2. Run the pump with an internal S50_70 waveform up to 100% amplitude. Record the maximum stroke volume. (should be 185 ish)
   Max Stroke: _____ mL/s
3. Stop the pump. Change the input waveform to External. Select a S50_70 waveform in ViViTest and hit apply.
4. Turn the pump off. Turn it back on and enter Calibration Mode. Enter Calibration menu 2. Turn the amplitude up to 100%. Write down the Max Stroke:
   Max Stroke: _____ mL/s
5. Adjust the External Gain value until the max stroke in step 4) matches the max stroke in step 2). Record the new External Gain value:
   External Gain: _____



18.4 Determining External Pump Calibration Value

1. Turn the amplitude to 0. Exit Calibration Mode. Repeat the following table using the external waveform:

Controller Stroke (mL/s)	ViViTest Stroke (mL/s)	= (ViViTest value / Controller Value) * 200
50		
100		
150		
Average:		

2. If different from the internal value. Average these 2 values together. Select the Pump calibration tab and enter the new value. Repeat entering the Acquire Tab of ViViTest process to save it.
3. Run both internal and external S50_70 waveforms at various amplitudes and ensure the stroke volume displayed by the controller matches the ViViTest value within +/- 0.1 or 0.2 mL/s

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19. Appendix G – Calculations

Crossover points are defined as follows	
F1 - Flowrate - Beginning of systole Forward Flow	P1 - Pressure Drop - Beginning of Systole Positive Pressure Drop
F2 - Flowrate - End of systole forward flow	P2 - Pressure Drop - End of Systole Positive Pressure Drop
F3 - Flowrate – Outflow Valve Closing, leakage begins	P3 - Pressure Drop - Beginning of Diastole Positive Pressure Drop
F4 - Flowrate - End of Leakage/Cycle	P4 - Pressure Drop - End of Diastole Positive Pressure Drop
(P) Calculated between Positive pressure markers (F) Calculated between Flow markers (H) Calculated between first Pressure and second Flow marker	

General parameters

Cardiac Output (Liters per Minute):
Mean Flowrate (F1 to F4)

Heart Rate (beats per min):
(60 (sec/min)) / Total Period (sec)
Total Period = total points between F1 to F4 / sample Rate (samples/sec)

Total Period (sec):
#samples between F1 to F4/sample Rate (samples/sec)

Flow parameters

Pump Stroke Volume (ml):
(Maximum Position signal – Minimum Position signal)* Cross-sectional Area of piston.

Calculated Stroke Volume (ml):
Cardiac Output (l/min) *1000 (ml/l) / Heart rate (beats/min)

Systolic percent of time
#samples between maximum and minimum position signal/# points between F1 and F4

Forward flow time
#samples between f1 to f2/ sample Rate (samples/sec) [F3 to F4 for Mitral]

RMS Forward Flow (ml/sec):
(RMS Flowrate (f1 to f2) *1000)/60 (sec/min) [F3 to F4 for Mitral]

Mean Forward Flow (ml/sec):
Mean Flowrate (f1 to f2) *1000)/60 (sec/min) [F3 to F4 for Mitral]

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Forward Flow Volume (ml):
Flowrate Area (F1 to F2)*1000(ml/Liter)/60(sec/min) [F3 to F4 for Diastolic FF volume]

Closing Volume (ml):
(flowrate Area (F2 to F3))*1000(ml/Liter)/60(sec/min) [F1 to F2 for Mitral]

Leakage Volume (ml):
(flowrate Area (F3 to F4))*1000(ml/Liter)/60(sec/min) [F2 to F3 for Mitral]

Regurgitant Fraction (RF) (%):
(closing volume + leakage Volume)*100/Forward Volume

Forward Flow Ratio (%):
Forward Flow time *100 / Sample rate (samples/sec)

Mean Leakage Flowrate (ml/sec):
-1* (Mean Flowrate (F3 to F4)) [F2 to F3 for Mitral]

Pressure parameters
Mean Pressure Drop [P] (mmHg):

(Mean of pressure difference between outflow and inflow of points between P1 to P2) [P3 to P4 for Mitral]

Mean Pressure Drop [F] (mmHg):
(Mean of pressure difference between outflow and inflow of points between F1 to F2) [F3 to F4 for Mitral]

Back Pressure (mmHg):
-1* (Mean Pressure Drop (F3 to F4))

Maximum Pressure Drop (mmHg):
Maximum Pressure Drop value between Points P1 to P2

Mean Aortic Pressure (mmHg):
Mean Aortic/Proximal/Pulmonary Pressure between P1 to P4 without compensation

RMS Aortic Pressure (mmHg):
RMS of aortic pressure F1 to F4 without compensation

Peak Aortic Pressure (mmHg):
Peak Aortic Pressure between P1 to P2 [Mitral between P3 to P4]

Mean Vent Pressure (mmHg):
Mean Vent Pressure between P1 to P4

Peak Vent Pressure (mmHg):
Peak Vent Pressure between P1 to P4
Parameters based on pressure and flow
E.O.A. (cm^2):
RMS Forward Flow (ml/sec)/51.6*(Square Root (Mean Pressure Drop/Density))

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Parameters based on Energy

Energy loss is calculated by integrating the flow times the transvalvular pressure over a relevant flow interval. A conversion factor of 0.1333 is applied to convert the energy from mmHg*ml to millijoules (mj).

Ventricular energy (VE) (mj):

Ventricular energy is computed using the following integral

$$\mathrm{VE} = \mathrm{Conversion\ factor} * \int_{\mathrm{F1}}^{\mathrm{F4}} \mathrm{P_{vent}} * \mathrm{DL\_Dt}(\frac{\mathrm{ml}}{\mathrm{s}}) * \mathrm{dt}$$

Where the integral is evaluated using trapezoidal rule and dt= 1/sample rate

Forward Energy (FE) (mj):

$$\mathrm{FE} = \mathrm{Conversion\ factor} * \int_{\mathrm{F1}}^{\mathrm{F2}} \Delta\mathrm{P} * \mathrm{flow}\ (\frac{\mathrm{ml}}{\mathrm{s}}) * \mathrm{dt}$$

Closing Energy (CE) (mj):

$$\mathrm{CE} = \mathrm{Conversion\ factor} * \int_{\mathrm{F2}}^{\mathrm{F3}} \Delta\mathrm{P} * \mathrm{flow}\ (\frac{\mathrm{ml}}{\mathrm{s}}) * \mathrm{dt}$$

Leakage Energy (LE) (mj):

$$\mathrm{LE} = \mathrm{Conversion\ factor} * \int_{\mathrm{F3}}^{\mathrm{F4}} \Delta\mathrm{P} * \mathrm{flow}\ (\frac{\mathrm{ml}}{\mathrm{s}}) * \mathrm{dt}$$

Total Energy (mj):

Forward Energy+Closing Energy+Leakage Energy

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20. Appendix H – Step By Step Guide

20.1 Definitions

Symbol	Definition
Feasibility test	A test conducted for research and development purposes only. This type of test is not intended for submission to a regulatory body.
Regulatory test	A test conducted with the intent of submission to a regulatory body (i.e. FDA, CE). The test should fully comply with all applicable regulations.
BPM	Frequency of operation expressed in terms of beats per minute.
SV	Stroke Volume is the amount of fluid ejected from the left ventricle during each beat. Value is calculated from piston area and stroke length.
CO	Cardiac Output in liters per minute (LPM) calculated as product of SV and HR.
FV	Forward Flow Volume is the volume (mL) of fluid passing through the valve during the forward flow phase of the cycle.
Regurgitation %	Total regurgitation expressed as a percent of the total forward flow [(Closing Volume + Leakage Volume)/FV].
Transvalvular Regurgitant Fraction	Is defined as the volume of fluid that flows through a valve in the reverse direction during one cycle and is the sum of the closing and leakage volume expressed as a percentage of the stroke volume. This assessment is conducted with sealing applied to the valve to prevent paravalvular leakage.
Total Regurgitant Fraction	Is defined as the volume of fluid that flows through and around a valve in the reverse direction during one cycle and is the sum of the closing and leakage volume expressed as a percentage of the stroke volume. This assessment includes both transvalvular and paravalvular leakage.
EOA	Effective orifice area determined by the Gorlin equation (cm^2)

$$EOA = \frac{Q_{rms}}{51.6\sqrt{\frac{\Delta p}{\rho}}}$$

Where:

$\mathbf{Q_{RMS}}$ is the root mean square forward flow (mL/s) during the positive differential pressure period or Aortic or Mitral RMS Flow [P] in ViViTest

$\Delta\mathbf{p}$ is the mean pressure difference measured during the positive differential pressure period (mmHg) or Trans Aortic or Mitral mean pressure [P] in ViViTest $\boldsymbol{\rho}$ is the density of the test fluid (g/cm^3)

Transaortic pressure	The ΔP across the aortic valve, the difference in aortic and ventricular pressures.
Transmitral pressure	The ΔP across the mitral valve, the difference in atrial and ventricular pressures.
AO Positive Pressure	The part of a cardiac cycle in which ΔP across the aortic valve is positive [ventricular pressure greater than aortic pressure].

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MI Positive Pressure	The part of a cardiac cycle in which ΔP across the mitral valve is positive [atrial pressure greater than ventricular pressure].
Test Fluid	The fluid that will come into contact with a sample in the Pulse Duplicator. Examples: Saline, Blood Analog, Bovine Blood etc.
VIA	Viscoelastic Impedance Adapter.
Companion Valve	The valve located in the opposite position of the valve being tested. For example if an aortic valve is being tested, the valve in the mitral position is the companion valve. By default the companion valve is the ViVitro Spring Loaded Disk Valve. **Note:** When possible, the companion valve in the mitral test position will be at least one size larger than the valve being tested in the aortic test position.
Sample	Could be valve etc. If the test specimen requires a holder to interface with the testing equipment the entire assembly will be considered a sample.
Run	The measurement, recording and subsequent averaging of required parameters for 10 cardiac cycles.
Nominal Conditions	Test parameters used for acceptance criteria listed in ISO 5840. Consisting of 5 LPM at a beat rate of 70/minute and stroke volume of 75 mL/stroke with a 35% systolic duration.

20.2 Summary Test (ref ISO 5840-3 Annex N)

The heart valves undergoing testing are mounted in the active test position (aortic/mitral) of the Pulse Duplicator and a companion valve is mounted in the opposite test position. The test loop is filled with a blood analog solution. The valves are then subjected to pulsatile flow operation that is intended to simulate physiologic conditions. Measurements of the various chamber pressures and the flow through the test position are obtained. The results are used to determine the pressure drops through the valve during the forward flow phase of the cycle and the reflux volumes of fluid during the closing and leakage phases of the cycle.

20.3 Cautions

- Do not turn flow meter on while system is drained. Ensure flow meter is always turned off before draining the system.
- When flushing pressure ports ensure this is done gradually to ensure pressure limits are not exceeded. Watch ViViTest to get a sense of pressures obtained while flushing.
- Never operate the Pulse Duplicator without ViViTest in live mode with pressure sensors connected and operating properly.
- When testing valves always make changes to the system gradually, if anything seems abnormal stop the system and re-evaluate.

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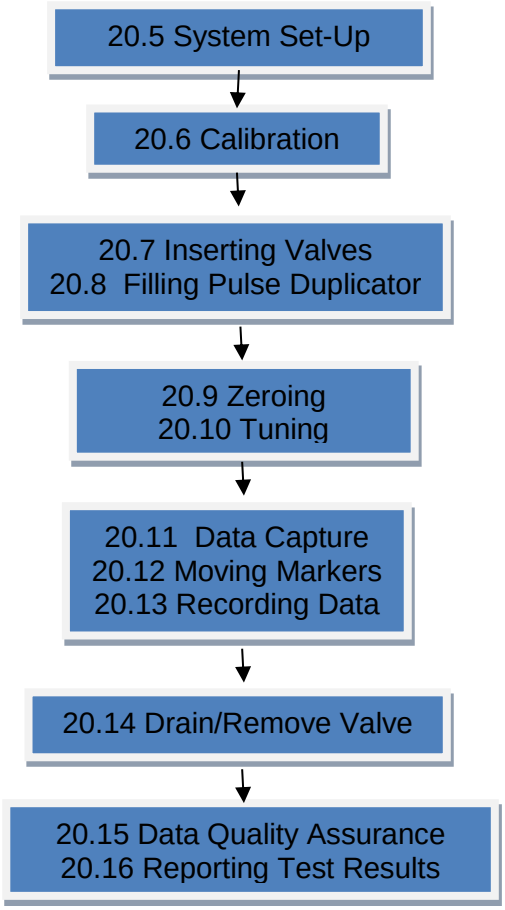
20.4 Pulsatile Flow Testing



20.5 System Setup

1. Set-up Pulse Duplicator per User Manual.
2. Mount flow probe in location desired for test, either aortic or mitral. Refer to Pulse Duplicator User Manual for instructions.

Note: Ensure that flow probe is oriented such that the side with numbers is the outflow side.

3. Turn on SuperPump controller, ampack, and flow meter to allow electronics to come up to operating temperature
4. If required turn on the Heat bath to allow it to come up to required temperature.

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20.6 Calibration

1. Start ViViTest software and open the calibration file that the software defaults to.
2. Click

tab.
3. Click

and follow prompts to take zero voltage reading of all channels.
4. Set up Pulse Duplicator for pressure calibration as per Pulse Duplicator User Manual.
5. Click

and calibrate as per prompts. Use a manometer for pressure reference, ensure that calibration is valid.
6. Set up Pulse Duplicator for flow calibration as per Pulse Duplicator User Manual.
7. Remove all air from VIA if installed.
8. Follow Flow Measuring System setup instructions as per Pulse Duplicator User Manual.
9. Click

and calibrate as per prompts.
10. If testing on a new system for the first time, or if displayed pump and ViViTest stroke volumes do not align
11. Click

and calibrate as per User Manual.
12. Click

and save calibration file as “YYMMDD_TP-XX-XX.cfg” (renaming is optional)
13. Turn off flow probe by turning “Probe” knob on flow meter to “Off“
14. To verify that the calibration was done correctly, ensure the flow meter is turned on and the pressure ports are open to the test fluid. The flow signal should read zero and all the pressure tracings should be at the same level as the ventricular pressure as shown below Figure 1.

Figure 1 - Calibration verification

15. Drain Pulse Duplicator according to Section 20.14.

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20.7 Inserting Valves

Do not insert bio-prosthetic valves without immediately filling system with test fluid. Handle valves per manufacturers instructions for use.

1. Insert the valve and holder assembly into the Pulse Duplicator as instructed in the User Manual. Note: Holders can be used with and without a step. If a holder has a step remove the keeper ring as listed in the Pulse Duplicator User Manual.

Note: It is recommended that post one is oriented towards the inside the of the Pulse Duplicator i.e. If testing an aortic valve ensure that post 1 is oriented towards the mitral chamber, this will ensure constant high-speed videography. If testing a bi-leaflet valve ensure axis between the leaflets is oriented towards the mitral chamber so gravity will have the same effect on both leaflets.

2. If necessary carefully insert outflow tract of valve into glass aorta
3. Install the companion valve as stated above.
4. For each valve record the valve's manufacturer, serial number, and size in the laboratory notebook as they are tested

20.8 Filling Pulse Duplicator

1. Add test fluid through atrial reservoir.
2. Begin pumping fluid by turning SuperPump up to a stoke volume of ~50ml/stroke.
3. Turn flow probe on by turning "Probe" knob on the flow meter to "+"
4. Continue to add test fluid as required.

Note: It is recommended that the height of the test fluid in the atrium should fluctuate about the same level during testing. This can be adjusted by either adding/removing air from compliance chambers or adding/removing test fluid. Use reference level marked on atrium as a reference.

5. Remove any air collected in aortic view port or atrium using overflow tube or atrial pressure port respectively.
6. Ensure pressure transducers are free from air bubbles. Flush with a syringe or bleed fluid from the system.
7. Add air to compliance chambers if necessary as this will affect the fluid level in the system
8. Confirm fluid level is appropriate as above
9. Run system until appropriate temperature is reached, measure using calibrated thermometer.

20.9 Zeroing the System

1. This is conducted before each data set is collected to account for any baseline drift on all inputs but most importantly to account for any flow meter drift.
2. Ensure system is at the desired testing temperature and has been running for ~5 minutes
3. The flow meter should be on the "+" on position
4. Ensure pressure transducers are free from air bubbles. Flush with a syringe or bleed fluid from the system.
5. Stop SuperPump

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6. Open pressure transducers to atmosphere using stopcocks
7. Allow fluid level to stabilize

NOTE: The live voltage readings should have fluctuations less than +/- 0.005 mV before proceeding.

8. Click

twice to take zero reading
9. The flow meter may experience a large drift, but all other values (Aortic, Ventricular, Atrial, dL/dt, and pump) should have a drift < 0.01 mV upon zeroing. If outside this range contact the study director.
10. Open pressure transducers back to fluid
11. Proceed to collecting data promptly to minimize flow meter drift

20.10 Tuning System

1. Depending on test plan, valves can be tested under a variety of operating conditions. The following outlines the steps to obtain the required testing parameters.
2. With pumped stopped set desired waveform and beat rate in ViViTest, click “apply”. See Figure 2.

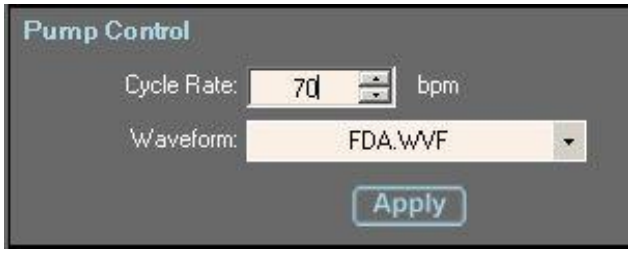

Figure 2 – Setting Waveform and Beat Rate

3. Increase amplitude on SuperPump controller to desired SV.
4. Visually inspect dL/dt waveform to ensure that appropriate waveform has been selected.
5. Remove any air collected in aortic view port or atrium using overflow tube or atrial pressure port respectively. Ensure overflow tube is closed before proceeding. Air will accumulate in the aortic view port each time the Pulse Duplicator is stopped and will need to be removed every run.
6. Use peripheral resistance knob to adjust either Mean Aortic Pressure or back pressure per the test plan.
7. Adjust root compliance such that test fluid is not rising up silicone tubing. If too much air is added, it will by-pass the aortic root and will need to be flushed according to step 5.5.
8. Adjust aortic compliance such that test fluid is rising and falling around the approximate half way level of the chamber. If testing at 100 mmHg the aortic pressure should be varying between 120 +/-10 and 80 +/-10 mmHg.
9. Adjust VIA compliance if specified in the test plan. Record any changed in lab notebook.
10. Stop the pump.
11. Re-zero system according to Section 20.9.
12. Repeat steps 20.10.3 through 20.10.9 to ensure desired conditions are still being met before proceeding with data capture.

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20.11 Data Capture

This section describes the process for testing a single valve. This will have to be repeated for each valve in the test plan.

1. Install test valve in Pulse Duplicator in position to be tested as per Section 20.7.
2. Fill system as per Section 20.8 allowing system to come to desired operating conditions
3. Fill in session information in ViViTest:
4. **Trial Title:** test plan number (TP-xx-xx)
5. **Trial Number**: 00 (optional)
6. **Aortic Valv**e: valve ID in aortic position
7. **Mitral Valve:** valve ID in mitral position
8. **Operator ID:** your name (optional)
9. **Ventricle Vol:** 150.00 ml (optional)
10. **Fluid Density:** 1.01 g/ml if saline is test fluid (or as specified in test plan)
11. **Fluid Temp:** 37 °C (or as specified in test plan) (optional)
12. **Flow Probe Site:** set to location to test valve
13. **Target Valve:** set to location of test valve
14. Record the following in lab note book, if not already done:
15. Test fluid used and relevant parameters if specified in the test plan (suggested viscosity and density)
16. Temperature of test fluid
17. VIA compliance volume
18. Companion valve used
19. Ensure test fluid is at desired temperature as specified in the test plan with calibrated thermometer.
20. Tune system to first desired parameter as per Section 20.10.
21. Once system has stabilized with desired parameters click

22. Once data is collected save in desired test folder using name “VALVE ID_RUNXX” (optional)
23. Follow Section 20.12 for aortic or mitral valve.
24. Repeat steps 20 for each test condition in the test plan.
25. If required by the test plan record high speed video of each valve at nominal conditions.
26. Once all conditions are complete proceed to Section 20.14 to drain the system and remove test valve.
27. Have the study director sign off after the first data collection.
28. Notify QA that a testing audit can be conducted.
29. Repeat above for each valve in test plan.
30. Once all valves have been completed proceed to Section 20.15 to ensure data quality.
31. Ensure data is being backed-up as appropriate.
32. Once testing is complete have study director sign off.

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20.12 Moving Markers

1. Once data has been captured the analyze tab will need to be used to extract the relevant flow parameters. The procedure differs whether an aortic or mitral valve is being tested.

Aortic Valve Testing

2. Clicking

will take you to the screen to analyze collected data. The software will automatically open the most recently collected data file, to open a different file use the navigator on the right side of the screen.

3. **Note:** When analyzing data in ViViTest, channels can be turned on/off by clicking on the desired channel in the legend below the pressure/flow curve display. This can make it easier to locate crossovers for maker setting.

4. Set "**AO Positive Pressure**" by setting "**ao–ve1**" to the point where **TRANSAORTIC** signal first rises above zero and "**ao–ve2**" to where it crosses back over zero, see Figure 3. *In other words move* "**ao–ve1**" to the first point at which the ventricular pressure is above the aortic pressure. Set "**ao–ve2**" to the last point at which the ventricular pressure is above the aortic pressure.

Figure 3 – Example of AO Positive Pressure

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5. Set "**V1"** [aortic forward volume] by moving marker "**f1**" to the point at which **Flowmeter** signal begins to rise above the zero line and marker "**f2"** to the point at which the **Flowmeter** signal crosses the zero line again, See Figure 4.

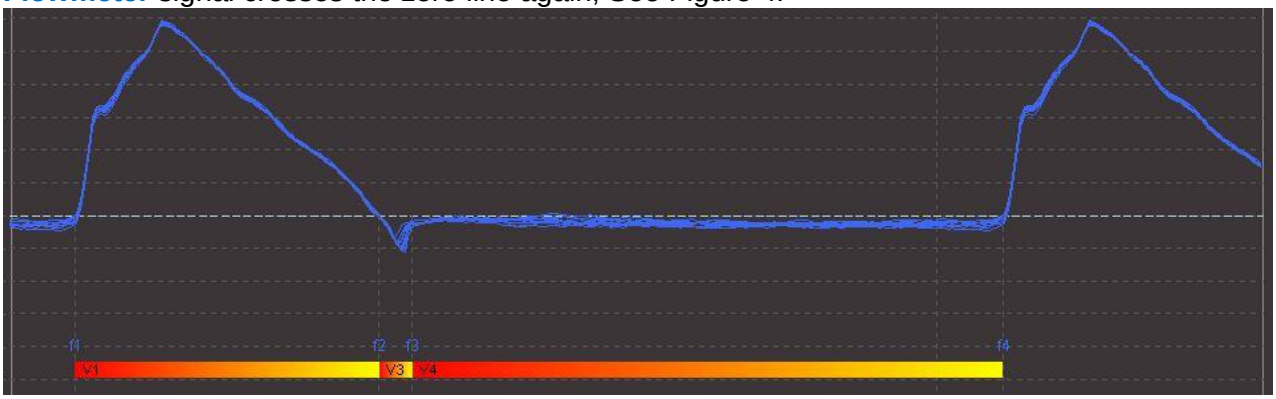
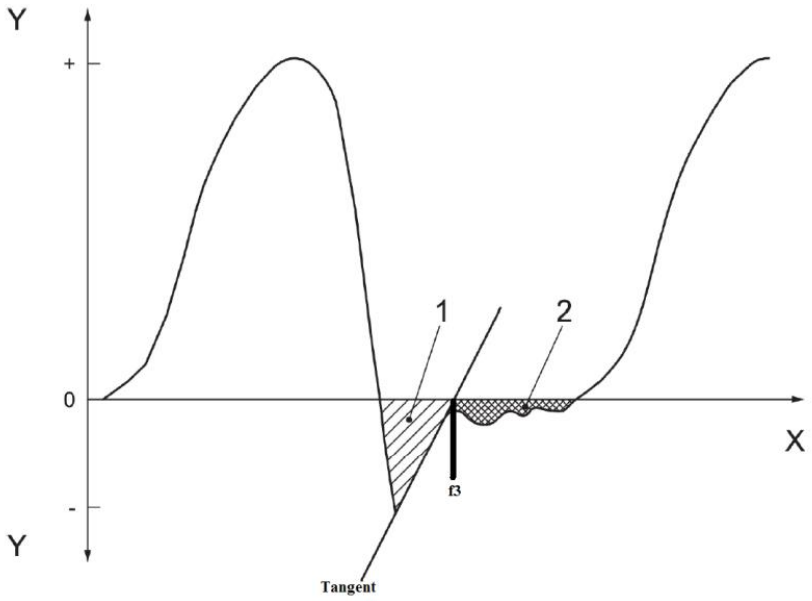


Figure 4 – Example of flow marker setting

6. The closing volume of a valve will usually be a sharp spike of flow in the negative direction. Create an imaginary line that runs tangent to the **Flowmeter** signal along the straight portion as it begins to rise back towards the zero line. Set "**f3"** to where this imaginary line would intersect zero if it were to be extended. See Figure 4 and Figure 5 for more detail. Markers F2 to F3 will create V3 or the closing volume.



Key

X	time
Y	flowrate
1	closing volume
2	leakage volume

Figure 5 – Example of closing volume (from ISO 5840)

7. Set "**f4**" to the point at which the flow begins to rise above zero at the end of the cycle. As this is the end of the cycle it should be similar to where "**f1**" was set but at the beginning of the next cycle on the display. Markers **"f3"** to **"f4"** will create the leakage volume V4 as shown in Figure 4.

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8. Once this is complete the data can now be recorded, proceed to Section 20.13.

Mitral Valve Testing

9. Clicking on the ANALYZE tab in ViViTest will take you to the screen to analyze collected data. The software will automatically open the most recently collected data file, to open a different file use the navigator on the right side of the screen,

10. **Note:** When analyzing data in ViViTest channels can be turned on/off by clicking on the desired channel in the legend below the pressure/flow curve display. This can make it easier to locate crossovers for maker setting.

11. Set "**AO Positive Pressure**" by setting "**at–ve1**" to point where **TRANSMITRAL** signal first rises above zero and "**at–ve2**" to where it crosses back over zero, see Figure 6. *In other words move* "**at–ve1**" to the first point at which the mitral pressure is above the ventricular pressure. Set "**at–ve2**" to the last point at which the mitral pressure is above the ventricle pressure.

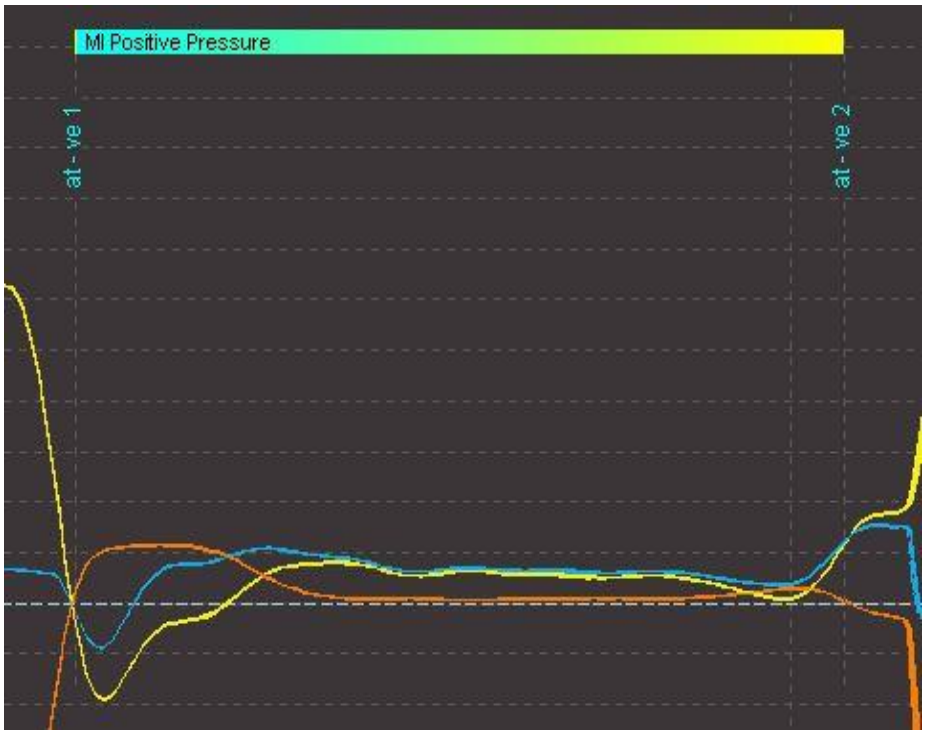

Figure 6– Example of MI Positive Pressure

12. Set "**V1"** [Mitral forward volume] by moving "**f3**" to the point at which **Flowmeter** signal begins to rise above the zero line and "**f4"** to the point at which the **Flowmeter** signal crosses the zero line again, See Figure 7.

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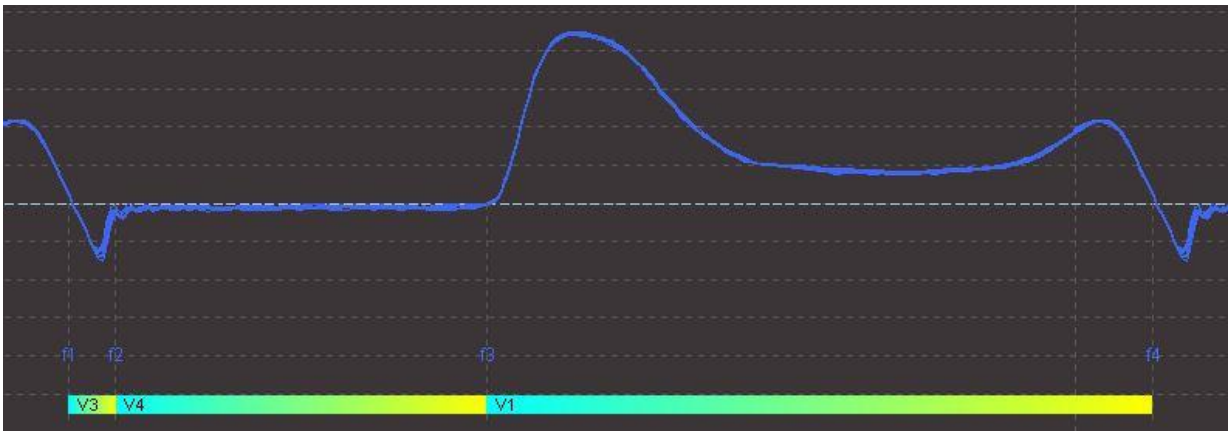

Figure 7– Example of flow marker setting

13. The closing volume of a valve will usually be a sharp spike of flow in the negative direction. Set "**f1**" to the point at which the **Flowmeter** signal first crosses the zero line on the left side of the screen. Create an imaginary line that follows the tangent of the **Flowmeter** signal along the straight portion as it begins to rise back towards the zero line. Set "**f2"** to where this imaginary line would intersect zero if it were to be extended. See Figure 5 and Figure 7 for more detail.
14. Once this is complete the data can now be recorded proceed to Section 20.13.

20.13 Recoding Data

1. Data should be recorded in the lab notebook in accordance with the lab's procedures.
2. Click the button to bring up the analysis table.
3. Click the button to save .ACQ file. Use the same file name as used in step 20.11.22.
4. Close analysis table.
5. Record the following information in lab notebook for each run of each valve:
6. **Run** – Begin at "RUN01" and increment for each test condition
7. If a run at the same condition has to be repeated keep the same number and affix a letter and increment as necessary. (example: RUN03B, RUN03C)
8. **Stoke Volume** (mL)
9. **Cardiac Output** (LPM)
10. **Mean Aortic Pressure** (mmHg) or **Back Pressure** (mmHg)
11. **Closing Volume** (mL)
12. **Leakage Volume** (mL)
13. **Regurgitant Fraction** (%)
14. **EOA** (cm^2)
15. **Waveform** (Sine50, Sine30, FDA etc.)

20.14 Drain / Remove Valve

1. Ensure flow meter is turned off before system is drained.
2. Open ventricle drain and move overflow tube to aortic drain port to speed up draining.
3. Ensure valve is removed promptly upon system draining.
4. Rinse valve if necessary before placing in container.

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20.15 Data Quality Assurance

This section is meant to identify potential procedural errors. Test samples may vary from the expected results below.

1. Forward flow performance (Runs 1-4)
2. Decreasing RF as CO increases (similar closing and leakage with increasing forward flow).
3. Constant EOA (possible deviation at low flow).
4. Forward flow pressure drop and Qrms plot should be linear.
5. 70 BPM and 5 LPM cases leakage should increase with backpressure and EOA should remain constant.
6. Regurgitant Performance
7. Closing volumes should be constant for a given beat rate.
8. Closing volume should increase with beat rate.
9. EOA should be constant at each beat rate set.
10. Leakage volumes should increase with increasing back pressure. However bio-prosthetic valves may seal better with increasing back pressure potentially reducing leakage.

20.16 Reporting Test Results

1. Create test report.
2. Include a snapshot from high speed video of each valve at both full systole and full diastole.
3. Include any relevant photos taken during valve inspection.

20.17 Maintenance

1. Follow instructions listed in User Manual
2. Disassemble all parts of the Pulse Duplicator, soak in 0.1% bleach solution, rinse, re-assemble, rinse and drain system with distilled water 3 times prior to use.

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21. Appendix I – End User License Agreement

The terms and conditions that follow set forth a legal agreement between you (either an individual or an entity), and ViVitro Labs (herein referred to as “ViVitro Labs”) with its principal place of business at 455 Boleskine Road, Victoria, BC Canada V8Z 1E7, relating to the computer software known as ViViTest and certain other related software modules, if applicable (herein referred to as "Software").

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ViVitro Labs warrants all parts of its own manufacture to be free from defects in material and workmanship under normal use and service in a suitable environment for the period of one year after delivery.

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22. *Appendix J* – Wetted Materials

The Pulse Duplicator consists of the following materials that are in contact with the test fluid:

- Acrylic
- Polycarbonate
- Buna-N
- Teflon
- ABS
- Platinum
- 316 SS
- PVC
- Silicone
- Acetal

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